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Supporting international safeguards

The US State Department must now persuade Congress to back the international safeguards system — and then worry about what will happen a decade hence.

Not before time, the United States is edging in the right direction in its policy on the proliferation of nuclear weapons. Almost two years have gone since President Reagan, then merely a candidate for his office, began promising that he would repeal President Carter's Nuclear Non-Proliferation Act, passed by Congress in 1978. Now, it seems, the Administration is planning to do what it can, within the framework of the act, to remove some of the sillier restrictions on the supply of nuclear equipment from the United States made necessary by this unfortunate legislation (see page 279). But there is still no sign of when it plans to carry the fight to Congress, seeking at least to amend the Carter act. Mr James Malone's departure from the State Department (*Nature*, 18 March) may be a sign of impatience, but mere irritation is not a substitute for action. Part of the trouble seems to have been that the Reagan Administration, while staunchly opposed to the Carter act on the doctrinal grounds that it needlessly restricted the freedom of American suppliers to sell nuclear equipment overseas, has not fully understood why the Carter act is self-defeating. Now, to judge from the good sense of what the State Department was saying last week about the problems of nuclear proliferation, enlightenment is at hand.

How can a piece of legislation with such laudable objectives have the opposite effect, of assisting the proliferation of nuclear weapons? The explanation, now familiar, is not all that complicated. Between 1965 and 1970, the United States took the lead in persuading non-nuclear powers to sign the Non-Proliferation Treaty, then offered as the best way of controlling the spread of nuclear weapons. Persuasion was necessary because, to many non-nuclear powers, it was not self-evident that a treaty that did not restrict the right of nuclear powers to keep on making nuclear weapons could serve the stated purpose. Against the odds, however, persuasion worked. More than a hundred non-nuclear powers have ratified the treaty and have agreed to put up with repeated and unavoidably irksome visits by inspectors from the International Atomic Energy Agency.

The Nuclear Non-Proliferation Act, especially as interpreted by the Carter Administration, was a shock to this system because it signalled that in the opinion of the United States, even strict compliance with the terms of the treaty would be insufficient. The act was also a unilateral denial of the promise in the treaty that the nuclear powers would help with the free flow of nuclear technology. And by implicitly asserting that compliance with the international safeguards system would not be taken as an assurance that a signatory of the treaty was not making nuclear explosives on the sly, the act undermined the credibility of the safeguards system as a whole. No doubt the consequences would have been more serious if the civil nuclear industry had not been in such doldrums in the past few years.

The new enlightenment that has overtaken the United States Administration, well put by Mr Eugene Rostow last week, is that technical devices such as the safeguards system operated from Vienna are necessary but not sufficient means for controlling the spread of nuclear weapons. Now, as since the invention of nuclear explosives, the decisive determinants of the spread of nuclear weapons are likely to be political — that is, governments' perceptions of the international dangers that confront them and their calculations, never straightforward, of whether nuclear weapons would help or, by provoking imitation, hinder. So

governments such as that of the United States seeking to limit the spread of weapons can hope to accomplish more by diplomacy than by tinkering with the Vienna safeguards. After all, the non-nuclear powers best placed to make nuclear weapons for themselves — India, Israel, Pakistan and South Africa, for example — are not even signatories of the treaty. In this sense, the preoccupation of the United States Congress (and of two unreflective members of the Nuclear Regulatory Commission) with the supposed loopholes in the safeguards system are strictly speaking irrelevant, and should be recognized as such. Luckily, the Administration seems to have accepted the point. Its task now is to persuade those who continue to grumble that even though it is not feasible to make the safeguards system strictly watertight, that does not imply either that the system is without value or that it should be scrapped.

No safeguards system can be perfect. The Vienna system now in force cannot, for example, guarantee that some signatory of the Non-Proliferation Treaty has not built clandestine nuclear plants in some remote part of its territory. (Surveillance satellites, however, can.) Nor can it prevent a government that has privately decided to make nuclear explosives from taking advantage of the intervals between physical inspections secretly to divert fissile materials to some hidden bomb-making plant. It is, however, unthinkable that any government would invite the trouble that would follow when it could achieve the same objectives by withdrawing from the treaty at three months' notice. Then even the best-designed monitoring instruments can break down, and the most alert inspectors be misled. The pursuit of perfection is therefore pointless. So long as the chance of the detection of violations of the rules is substantial, the international community has a reasonable assurance that signatories of the Non-Proliferation Treaty are not breaking the rules.

None of this implies that the safeguards system needs no attention. But its most serious weaknesses are not technical but administrative and financial. The new director-general of the international agency, Dr Hans Blix, has been stressing in the past few weeks that the technical qualifications of his small but rapidly growing staff of inspectors should be improved. But where will these people come from? As things are (or were at the end of 1981), 130 qualified inspectors were required to inspect a total of 850 nuclear installations, but were able to carry out only half as many inspections as they should have done. What will happen when the number of nuclear installations under safeguards has grown by an order of magnitude, and when inspections will have to be more frequent because the quantities of fissile material involved will be much larger? On the face of things, the international inspectorate will need several thousand skilled people. Is it sensible to think that they will materialize, or that their work could be organized effectively?

The danger that at some point in the future the safeguards system will collapse under its own weight is thus perhaps the most serious threat to the integrity of the whole system. What can be done to head it off? In the short term, technology has much to offer. Automatic devices for monitoring what happens in nuclear plants have been installed within the safeguards system, and will no doubt be improved (see page 279). Devices that could sample data from a distance would be potentially invaluable. There is also some reason to believe that inspection could be simplified if those



who operate reactors, for example, would agree that a continuous and incorruptible record should be kept of, say, neutron flux or some other physical quantity sensitive to the movement of nuclear material within them. The safeguards system now in use, devised merely to account for quantities of fissile material in the input to and the output from a reactor, does not require the collection of such data — which would in some places be regarded as an infringement of national sovereignty. It is not too soon to plan for a return of this provision at the next meeting of the signatories of the treaty three years from now.

Further ahead, there is a strong case for planning for a much more radical simplification of the safeguards system. Even as things are, governments with nuclear installations on their territory will, if they are prudent, take steps to make sure that fissile material is not spirited away. These domestic interests thus coincide with those of the international safeguards inspectorate, but the work is duplicated. The ideal, then, would be that self-policing should be done in such a way that it could be unambiguously and internationally verified. (Some concessions to this notion are already made within the Euratom system, which has its own set of safeguards.) The sooner this goal is recognized, the more likely it is to be attained. And that, in the long run, will be the best assurance that this important instrument in the non-proliferation system remains intact.

Problem not for now

Is it too soon to be worrying about who should operate the shuttle system?

If this week's space shuttle, diverted by the aftermath of heavy rain from California to New Mexico, returns successfully, the chance that it will become a white elephant will substantially have diminished. The first two flights have shown that the machine will function as intended. This time there should be information bearing on its potential usefulness. But it will be a long time before anybody will know that the shuttle will do the job for which it has been built — to put large satellites cheaply into orbit. The immediate need is to reduce the intervals between successive flights of the one machine in service — an interval artificially lengthened by the need to return it from its landing site to its launching pad in Florida but also by the need to replace whatever ceramic tiles have fallen off in flight. The plan is that the next flight but one should take off from and land in Florida, but only when there are four machines in service will it be possible to tell whether the turn-round time is really as short as planned.

So why is the United States government already in a tizzy trying to decide within what legal framework the shuttle should be operated in the closing years of this decade? It is not even as if the problem is all that novel. The development of telecommunications satellites in the 1960s is an obvious precedent. Then, as with the shuttle, there were three kinds of customers in sight — the US Department of Defense, potential users of communications satellites in the United States and customers from elsewhere, principally the international consortium of communications authorities called Intelsat. The largely successful solution was to leave the launching of military satellites to the Pentagon and to set up the corporation called Comsat as an organization independent of the government for launching and managing communications satellites.

Operating the shuttle commercially will be more complicated only in two respects. It may turn out to be uneconomic to have separate spacecraft for civil and military launchings, while, for a time at least, whoever owns the first four spacecraft will enjoy a monopoly of some kind. But there is no reason why a corporation along the lines of Comsat should not occasionally work for the military, and no reason why the temptation to make outrageous profits should not be restrained by a modicum of regulation. These difficulties will be clarified only several years from now. In the meantime, it might be thought, the US government has more urgent problems crying out for its attention.

Europe in the doldrums

Could science and technology help the EEC to a second twenty-five years?

What is to become of the European Community, which will be celebrating its twenty-fifth anniversary this week? On recent form, member governments will use what energy they can spare for the Community on now-familiar disputes about the prices that farmers should be paid for various foodstuffs or the shares to which they are entitled from this and that central fund. In their defence, governments will say that in the middle of a recession and with more than 10 million people unemployed in Europe, this cannot be the time for pausing in the pursuit of self-interest. They will also rightly say that a great deal has been accomplished in a quarter of a century. There is a customs union which works reasonably well. The Community has been enlarged (from six to ten, but Greece is shaky). And there is a sense that Europe is culturally more of a piece than it was. The snag is that the benefits are intangible, so that it is the public quarrels that stick in people's minds.

That things should have come to such a pass is easily understood. The treaty signed in Rome on 25 March 1957 was necessarily a blend of idealism and practical politics. The earlier collapse of the plan to set up a European Defence Force had warned the negotiators that even the tiniest infringement of national sovereignty would have to be made explicit and agreed in advance. Although there has recently been some talk of concerted action on defence the European Community is unlikely to be chosen as the vehicle.

The tentativeness of the past quarter of a century means that even now the Community does not enjoy economic cohesion. While private companies are required to compete with each other freely, governments can and do bias their purchases in favour of their own national suppliers, thus denying all members of the Community the benefits of scale and of an economic division of labour in some of the most important fields of technology. There was a minor sensation when British Telecom ordered new exchange equipment from a non-British corporation, but nobody appears seriously to have suggested that the British Central Electricity Generating Board should order the pressurized water reactor it wants to build from say Framatome (see page 299). Yet the European Commission in Brussels is wringing its hands over what used to be called the "technology gap" and seeking some way of strengthening the industries that its members have themselves weakened by their purchasing policies. Would it not be more productive to work out some set of inducements for persuading the member governments that they must give up their technological chauvinism?

Much the same question should soon be asked about the support of Community governments for research. Over the past quarter of a century spending by the European centre on research has if anything been set back. At the outset there was Euratom, but now there is merely the Joint European Torus and a miscellaneous programme of research at the old Euratom laboratory in Italy. Otherwise, governments deal independently with their spending on research, making separate decisions about their membership of international organizations or their spending in their domestic laboratories.

Up to a point, all this is justifiable. Governments responsible for universities also have to equip them for carrying out research, but even here there is scope for planning on a European basis complementarity between the centres of excellence that different governments support. Elsewhere, it is shocking that so little has been done to coordinate research on problems or fields of common interest. Should not, for example, something be done to concert the very considerable efforts in agricultural research, not so as to save money but in the hope of becoming more effective? The stock answer, that coordination works only inefficiently, is another way of saying that Europe is better balkanized. It would be better to devise machinery that made efficient collaboration possible.

US backs nuclear safeguards

Rostow hints at new policy on plutonium

Washington

The Reagan Administration rallied behind the International Atomic Energy Agency (IAEA) last week, claiming that a strong international safeguards system was essential for the development of nuclear commerce. It argued that weaknesses in the safeguards system should be remedied by greater support for IAEA programmes, not by changing their basic objectives.

The Administration's views were given in testimony to two subcommittees of the House of Representatives' Foreign Affairs Committee. One issue was the adequacy of the Administration's proposed contribution to IAEA in the 1983 budget. Although the increase over the 1982 contribution is larger than that for any other international organization, it will still not be enough to keep up with inflation — a source of concern to some State Department officials faced with rapidly growing demands for IAEA inspection procedures.

The hearing was also an opportunity for the State Department to rebut some recent criticisms of IAEA, in particular complaints about its admission last year (in connection with a reactor in Pakistan) that it cannot always assure member countries that nuclear materials are not diverted from peaceful to military purposes.

Such criticisms, State Department officials argued last week, are not only misdirected but also potentially harmful, tending to undermine the credibility of IAEA. Mr Richard T. Kennedy, Under-Secretary of State for Management and head of the delegation to IAEA, angrily rejected charges by Congressman Richard Ottinger that the agency had been involved in a "cover-up" by not making public its concern about the possibility of the diversion of nuclear material in countries such as Iraq and Pakistan.

Mr Kennedy was accompanied at the witness table by Ambassador Richard Kirk, deputy US representative to IAEA, and Dr Eugene Rostow, head of the Arms Control and Disarmament Agency. Each spoke strongly on the theme that the agency requires as much support as possible from the industrialized nations, and that the adequacy of safeguards should not be considered in isolation, but merely as one element in the control of nuclear proliferation.

Thus Mr Rostow told the subcommittees that halting the spread of nuclear explosives was "inconceivable" without IAEA safeguards, but added that they did not prevent diversion since, for example,

they did not permit searches for clandestine materials or facilities. "In my view, it is just as wrong to overestimate the importance of safeguards in nuclear commerce as it is to denigrate the system for not accomplishing objectives for which it was not designed," Mr Rostow said.

The State Department's consensus on IAEA, however, was not shared by all members of the Nuclear Regulatory Commission (NRC). Under the terms of the Nuclear Non-Proliferation Act of 1978, the commission is responsible for checking that safeguards are applied to any foreign nuclear installation to which nuclear materials are being exported from the United States.

Mr Peter Bradford, on his last day as one of the five members of NRC, was outspoken about the difficulties experienced by both NRC and congressional committees in obtaining data by which to assess the effectiveness of the safeguards. At one point, he accused the State Department of unnecessarily censoring NRC's reply to questions submitted by Congressman Richard Ottinger.

Mr Kennedy refuted the charge of censorship, pointing out that the information was being withheld at the request of the Central Intelligence Agency — which has since offered to brief Mr Ottinger on the subjects that he had inquired about. Both he and Dr Rostow, however, declined to say whether the State Department has evidence of the diversion of nuclear materials.

Both Mr Bradford and a second NRC

commissioner, Mr Victor Gilinsky, expressed reservations about the adequacy of IAEA inspection procedures for warning about the diversion of weapons-grade plutonium from reprocessing or enrichment facilities — concerns which led the Carter Administration to attempt to dissuade other countries from adopting such technologies.

Dr Rostow in reply criticized the previous Administration's approach, suggesting that attempts to impose unilateral controls could backfire by encouraging the spread of reprocessing while making less likely the agreements on a common policy with other nuclear suppliers. The Administration's policy is soon to be defined in a new executive order which Mr Reagan is to sign; Dr Rostow said it was important to acknowledge that civil reprocessing in the stable industrial democracies did not in themselves present a proliferation risk.

On the IAEA safeguards, Dr Rostow urged that member states should provide the international safeguards system with the resources needed. Mr Kirk, however, told the subcommittees that as the number of installations under IAEA safeguards had risen from 560 in 1977 to 850 in 1981, even though the IAEA budget allowed much faster growth for safeguards than in other activities, its expansion of the safeguards system had caused "a resources pinch, growing pains in IAEA's administrative structure, and a lag in IAEA safeguards coverage".

David Dickson

Nuclear monitoring by telephone

Washington

A scheme for collecting nuclear safeguards information by means of telephone lines is to be discussed at a meeting planned for Vienna in June this year. The system, called the Remote Continual Verification programme (or "RECOVER"), which was given a systematic trial in the autumn of 1980, has grown out of the technical proposals for the remote verification of arms control agreements in the draft of the Comprehensive Test Ban Agreement, uncompleted since the end of 1980.

In evidence to the House of Representatives last week, Dr Eugene Rostow said that on the basis of a cost-benefit study carried out at Brookhaven National Laboratory, the Administration was now hoping that the system could be put into service soon, and that it might even be valuable in the verification of treaties (yet to be negotiated) on chemical and biological weapons.

In the nuclear context, the new system is a means of making sure that automatic monitoring equipment does not break down between visits by inspectors from the

International Atomic Energy Agency (IAEA). This is done by the repeated but irregular interrogation of monitoring equipment by means of signals transmitted on the international trunk telephone system. So that the authorities responsible for safeguarded nuclear installations cannot corrupt the signals received and sent, signals are encoded by means of an unbreakable code of the type developed for use in what is now called public-key cryptography.

The Brookhaven study has apparently shown that the new monitoring system is potentially most valuable in nuclear installations such as reactors which can be refuelled on load and in fast critical assemblies, to which frequent visits from inspectors are at present required. One critical assembly in Japan, containing a fixed quantity of 300 kg of plutonium and 200 kg of enriched uranium, has on present criteria to be inspected every week or two.

The new monitoring system, by reducing the frequency of inspections, would save an estimated \$200,000 a year at that installation alone. The system is, however,

unlikely to be used at separation plants (where inspectors are virtually permanently in residence) or in light-water reactors (where redundant monitoring equipment is probably cheaper).

In Japan, the system is also being advocated as a means of safeguarding the sea transport of fissile material, providing a means of making sure that cargoes are not illicitly diverted on the high seas. In such an application, communication would be by means of Earth satellites of the kinds being developed for marine navigation. The effective use of the system for the inspection of nuclear installations on land is thought to require the development of a network of IAEA field stations (of which there are at present only two, in Toronto and Tokyo) and the existence of an efficient trunk telephone system (found wanting in Bulgaria during the 1980 trial of the system).

The application of the system to the monitoring of agreements on chemical and biological weapons presupposes the design and installation of effective automatic monitoring equipment at plants covered by an agreement.

Soviet nuclear power

Signs of caution

The Soviet nuclear energy programme is running into difficulties. In spite of the high priority given to power station construction, last year's targets were not met. And although no specific reference has been made to the need for greater standards of safety, Pavel Falaleev, first deputy Minister of Energy and Electrification, has said that the safety requirements of power stations were being tightened despite the radiation around them being "considerably below the permissible limit and practically no different from natural levels". In a Soviet context such a remark is sufficient to indicate considerable high-level rethinking.

The discussion of the nuclear programme began about six weeks ago, with a meeting of the Central Committee of the Communist Party to review shortcomings, where the emphasis was on the logistics of construction although inadequacies in the design sector were noted.

Three weeks later, the theme was taken up in a leading article in *Pravda*. This at first followed the line of the central committee meeting, noting that production-line nuclear power units had already been developed, and that supply problems could be dealt with by economic sanctions to penalize those who held up the plan. *Pravda* then went on, however, to suggest serious deficiencies in the design sector.

Here, it was claimed, the Ministry of Energy and Electrification had failed to exercise its supervisory duties. Changes in materials and specifications had been made without either proper justification or the

Sticky problem over Iraq fuel supplies

Two French nuclear physicists, backed by three prestigious members of the Academie des Sciences, have warned President Mitterrand in a report that there would be no easy way to stop Iraq making bomb-grade plutonium — if France rebuilt the Osirak reactor destroyed in a bombing raid by Israel nearly a year ago.

This is the second report condemning the reactor sale to be prepared by the two physicists and to be sent — unsolicited — to the president who is said to be sympathetic to the arguments but to be short of apolitical technical advice.

What stung the physicists to produce a second report were widely-reported claims that "caramel" — a low (7 per cent) enriched uranium oxide fuel — was the answer to the problem. If caramel were sold to Iraq to fuel the reactor, in place of the 95 per cent enriched uranium for which it was originally designed, the claims went, Iraq would have no quick route to the atomic bomb.

However, this misses the point, the new report stresses. The caramel fuel would still produce the same neutron intensity in the large pool around the reactor, where test materials are placed to investigate their reaction to neutron bombardment. If these test materials were replaced by depleted — or natural — uranium (of which Iraq is believed to have supplies) plutonium would be produced in the uranium, and could be extracted chemically.

The only advantage of caramel is that it cannot be used directly to make bombs, whereas 95 per cent enriched uranium is more easily converted. But, the reports point out, four years ago Giscard d'Estaing set limits on deliveries of enriched fuel to Iraq. Fuel would be sent in single reactor-loads of 13 kg, each of which would be loaded into the reactor under supervision and promptly irradiated making diversion technically very awkward. From that time, the diversion of the fuel itself ceased to be a problem — so the use of caramel solved nothing.

The production of plutonium by neutron bombardment, however, could amount to 3.3–8 kilogramme per year (to quote the assessments of both the Commissariat à l'Energie Atomique and the International Atomic Energy Agency). The amount of plutonium required for a bomb is generally taken to be 6 kg, although under certain circumstances 1–2 kg could be enough. Thus, say the two reports, Iraq could have the capability (in plutonium, at least) of producing a bomb within six months of beginning irradiation, whatever fuel the reactor was loaded with. IAEA inspection, however, might interfere considerably with this rate of production.

France has said it will help rebuild Osirak, provided Iraq guarantees that the reactor will be used entirely for peaceful purposes. The new report may make this politically more difficult for the president.

Robert Walgate

apparatus for selecting the "most progressive" solution to engineering problems. In particular, a proposal put forward by the *Atomenergostroyproekt* design trust, for a change in the structure of the "protective shells" of power stations, was turned down without being studied by the necessary multi-disciplinary panel of experts. The scientific council of the ministry, *Pravda* said, must bear greater responsibility for such decisions.

This reference to protective shells is significant. If, as seems likely, this refers to the concrete containment vessel common in Western power stations, it reflects a change in Soviet policy. Until recently, such vessels were dismissed by Soviet designers as unnecessary, and a capitalist ploy to raise construction costs. Demands from the Finns and Hungarians led them to introduce containment vessels into reactors designed for export. The remark in *Pravda* suggests that they may now have been introduced into reactors for home use whereas previously it was thought sufficient to surround reactors on the outskirts of major cities with a kilometre or two of parkland or playing fields.

Vera Rich

Intelligence testing

Soviet inequality

The Russians have finally "come out" on the subject of intelligence and other objective tests of performance and personality. The January/February issue of *Voprosy Psikhologii* (Problems of Psychology) contains three papers endorsing such tests in principle.

The papers are printed prominently at the front of the journal, preceded only by a major statement on future psychology services policy, based on the decisions of the November plenum of the Soviet Union's ruling communist party. The journal quotes President Brezhnev himself in support of a call to concentrate on "scientifically-based solutions to the problems of the nation's education in the service of the scientific and technological revolution".

This is a startling but unequivocal *volte-face* by the Soviet authorities, who have until now (in public at least) regarded "testology" as an instrument of class warfare in the hands of the ruling capitalist elite. Testing was even made illegal in

Russia in 1936 by an edict that may now have to be hastily repealed.

One of the most extraordinary admissions in the first paper, by K.M. Gurevich, is that there are still significant class divisions inside Soviet society, as well as national cultural differences within the empire, which test content must take into account.

Western workers in the field of intelligence testing may also prick up their ears at the news that foreign tests are already in use (presumably in the enforced absence of nationally-devised and standardized ones). In fact, it was the use of such tests which originally brought testing into disrepute in the Soviet Union. Gurevich advises: "Above all, when embarking on a study with one of these (foreign) tests, it is vital to decide what contingencies it makes sense to employ in order to make it an appropriate instrument for carrying out an investigation under conditions very different from those under which it was created". A citation at the end of his paper shows that Gurevich recently published a book on the subject (*Psychological Diagnostics. Problems and Research*, Moscow, 1981).

In the second paper, V.I. Slobodchikov makes an interesting distinction between "suitable" uses of test procedures — that is, they may be used for discrimination and selection but not for "controlling development or in remedial education".

At the opening of the third paper, by Yu. Z. Gil'buch, the British intelligence tester Professor H.J. Eysenck is accused of epitomizing the "mechanistic" attitude to testing. This does not deter Gil'buch, head of a Kiev laboratory, with more than twelve

"WHAT I CAN'T UNDERSTAND
ARE ALL THE HIGH
I.Q.S IN SIBERIA."



years of published work on the subject, from concluding his piece thus:

"The fundamental question at the root of any discussion about content validity in intelligence tests is this: what underlies individual differences in the degree of mastery of mental operations as exemplified in any particular culture (as represented in tests)? Is it mostly factors of biological inheritance, innate gifts, or is it (alongside and in interaction with these) the conditions of education and upbringing, which in various families and various schools are unavoidably present in greater or lesser variety?"

Soviet test experts are frantically trying to

devise tests which allow for different norms within classes and national cultures, but which will also serve the practical purpose for which the tests have been revived — namely, the more efficient selection of personnel to man their scientific and technical revolution.

The present aim of Soviet psychologists seems to be to devise tests which tap the purest, least culture-bound workings of the brain, such as solving abstract puzzles, chess problems and devising imaginary games.

Just how important this is to the Soviet Union is summed up by Gurevich thus: "In our country at present the growth of psychological diagnosis has become one of the most vital supports of theoretical and applied studies in the field of education, farming and management. In pre-school education, high school and professional training, methods of psychological diagnosis must be used to assess levels of psychological development: this allows for the inevitability of change within the very process of education, and provides a broad base from which it can advance to rationalization and improvement."

Elizabeth Roberts

Biological warfare

Soviet use

Washington

The Reagan Administration claims that it now has clear and scientifically verified data that the Soviet Union is providing toxic agents for military use by its allies in Laos and Kampuchea, and has circumstantial evidence that the Soviet Union is itself using such weapons in Afghanistan.

The evidence is contained in a report presented to Congress by Secretary of State Alexander Haig last Monday, providing a detailed analysis of eyewitness reports, chemical analysis of samples and information from other sources gathered over the past seven years.

According to Under-Secretary of State Walter Stoessel, the evidence shows that the Soviet Union has been engaged with its allies in the use of weapons that are forbidden by the Biological and Toxin Weapons Convention of 1972. "The USSR is flagrantly and repeatedly violating international agreements, and this is now a threat to the whole international community, since toxin weapons are a cheap, convenient way of subduing and exterminating opposition which could be used against other people", Mr Stoessel said.

The State Department's report is intended to stem sustained criticism that it has failed to substantiate its allegations of the use of chemical and toxin weapons — and in particular of tricothecenes — with adequate medical and scientific data.

Various other hypotheses have been put forward to explain the presence of tricothecenes in samples which have been brought back from South-East Asia and

Incriminating data

Washington

The State Department report details how the evidence for the occurrence of mycotoxins has been obtained. It says that the US Army's Chemical Systems Laboratory was unable to detect them in the few samples returned from South-East Asia, so that Dr Chester J. Mirocha from the University of Minnesota was asked to apply his gel-separation and mass spectrometric techniques to the problem.

Three closely related mycotoxins are said to have been identified: T-2, nivalenol and deoxynivalenol. A sample of material thought to be contaminated and obtained from Kampuchea contained 109 p.p.m. of nivalenol, 59.1 p.p.m. of deoxynivalenol and 3.15 p.p.m. of T-2. Control samples of vegetation, submitted for analysis gave negative results.

Samples of water from Laos and Kampuchea contained 150 p.p.m. of T-2 and 25 p.p.m. of diacetoxyscirpenol, another closely related toxin. A sample of yellow powder collected after a supposed attack by chemical weapons showed no evidence of toxins but did yield a yellow pigment, similar to one found by Dr Mirocha in a culture of *Fusarium roseum*. The report says that this may mean that the agents used in South-East Asia are crude extracts of *Fusarium* cultures.

A crucial element in the State Department's case is that the concentrations found in the samples from South-East Asia are greater than those associated with natural contamination. Typically, the latter yield only a few parts per million of mycotoxin, although one measurement of 41 p.p.m. of mycotoxin in contaminated grain in the United States is on record.

The report includes names of Soviet scientists and of four laboratories thought to be involved in research with mycotoxins. The laboratories are the Institute of Experimental Veterinary Science (Moscow), the Institute of Microbiology and Virology (Kiev), the Institute of Nutrition (Moscow) and the Institute of Epidemiology and Microbiology (Moscow). There is no suggestion that the research there is military in character.

analysed in US laboratories. Some have claimed, for example, that they could have come from various forms of rat poison, others that they might be the residue of naturally occurring fungus.

State Department officials, however, said at a press conference on Monday that they had looked closely at the various alternative explanations and had not been able to substantiate any.

Mr Richard Burt, the department's

director of political and military affairs, pointed to extracts from an East German military manual printed in the report which gives details of how toxin weapons might be used, pointing out that the suggested circumstances were similar to those reported in South-East Asia.

"We think Laos, Cambodia (Kampuchea) and Afghanistan are 'proving grounds' for testing the chemical and biological weapons capability of the Soviet Army", Mr Burt said. "In all three countries there is strong local resistance which stands in the way of Soviet objectives, and where the conventional use of troops would be very costly."

Department officials refused, on national security grounds, to say how all the various samples and reports had been obtained. In analysing the samples, Mr Burt said, the department had received technical cooperation from the British government as well as the Japanese.

Disputing press reports that tricothecenes were not powerful enough to be a useful tactical weapon, Dr Sharon Watson of the Army Surgeon General's Office said that experiments carried out at the army's testing centre in Fort Detrick, Maryland, had shown that haemorrhaging, caused by a severe impairment of blood-clotting, could occur at very low exposure levels. Experiments had shown that for a 70 kilogramme man the LD50 dose could be as low as 35 milligrammes.

Furthermore, Dr Watson said, the army had reason to believe that a crude extract from *Fusarium* was being used, which could be more toxic than the purified form. Referring to the broader implications of the charges being made against the Soviet Union, Mr Burt said that the evidence for the use of toxins in South-East Asia illustrated that one of the major flaws in the 1972 convention banning the use of toxins in war was that it contained no provision for verifying compliance.

David Dickson

Information technology

Cables coming

If Britain does not prepare to accept cable information systems now, then it may as well not bother at all. That is the message contained in a report to the Cabinet Office which was prepared by the government's Information Technology Advisory Panel and published earlier this week. Such is the urgency perceived by the panel of the need to provide cable television for British viewers that the report urges the government to make its intentions clear even before fully resolving some thorny problems such as regulation and licensing of the programmes that can be transmitted.

The government seems set to take on board the gist of the report's recommendations which include announcements of broad policy by mid-1982, regulatory arrangements by early 1983 and the

formulation of technical standards for the cable network by the end of this year. Civil servants in several government departments are now trying to work out the details. Thus the more leisurely approach to cable television which was envisaged as recently as early last year, when the Home Secretary approved 13 pilot schemes, looks like being abandoned. Decisions must now be taken, according to the report, before the results of those schemes can be known, in order to prevent overseas companies from hastening the decline of the British cable television industry.

The chief interest in cable systems is said to lie in their potential for linking new information technologies. But the panel believes that large numbers of users will only be attracted to the system quickly if it starts by offering a wide choice of television programmes. Mr Charles Read, chairman of the panel, hopes that programme providers can be licensed within existing legislation, which gives the Home Secretary wide powers of discretion.

Public fears about the quality of broadcast programmes, which traditionally have been tightly controlled in Britain, may not be so easily allayed. This problem will soon be tackled by Lord Hunt, formerly secretary to the Cabinet, who is to hold an urgent inquiry into the likely effects of cable television on the public broadcasting system.

Precise specifications for the cable system have yet to be established. But the panel's report envisages series of local networks, initially in large cities, that would offer broad bandwidth communications capable of carrying 15-24 channels. British packet-switching technology that would allow cable to individual homes to be of lower bandwidth than that on trunk lines is favoured.

The cost of cabling half the British population, according to the report, would be about £2,500 million, all of which would have to be found by the private sector, which apparently is eager to put up the money.

Costs, says the report, might be minimized if British Telecom ducts were used for laying the new cable. Local networks could, for example, be interconnected via its own telephone network. And as private broad bandwidth cables may well be installed before its own network is upgraded, the company would be wise to consider putting some of its own services, such as Prestel, onto the new cables.

While welcoming British Telecom's interest in the new cables systems, the panel's report is nevertheless cautious about the extent of the company's involvement. It is particularly keen, for example, that British Telecom should not dictate the standards for the new networks, presumably fearing that the company would be too restrictive and cause unnecessary delay.

Judy Redfearn

Neuroscience moves

New York

The Neurosciences Research Program, the seemingly clubby survival from the time when it seemed necessary, twenty years ago, to persuade people that neurobiology was interesting, is in the throes of moving from Boston to New York. At the same time, it has been given a new image and an income that it can call its own.

The organization was begun in 1962 by Professor Francis O. Schmitt, largely to proselytize on behalf of the neurosciences. Since then it has been housed in a replica of a French chateau 15 miles from Boston, and has been best known for its periodic small workshops (up to six a year) on various aspects of neurobiology. Now, however, the organization has negotiated a lease with Rockefeller University that will allow it to house not merely its administrative staff but a new institute, called the Neurosciences Institute, intended to provide up to half a dozen relatively senior people in the field (and perhaps as many junior colleagues) with an opportunity for conceptual (as distinct from experimental) work for short periods of time.

Both parties to the lease now signed are anxious to emphasize that the Neurosciences Research Foundation, by becoming a tenant of the university, will not become a part of it. The foundation will in future finance both the Neurosciences Institute and the Neurosciences Research Program from an income that appears to exceed \$500,000 a year.

In the move from Boston to New York, some care seems to have been taken to broaden representation in the management of the enterprise, with the result that it is often hard to tell who will do what. The director of the Neurosciences Research Program from the president of the foundation) is Dr Vernon B. Mountcastle, President of Johns Hopkins University. Dr W. Maxwell Cowan of the Salk Institute is the chairman of the Scientific Advisory Committee of the programme, but there is also a "scientific" chairman, Dr Gerald M. Edelman of Rockefeller University (who is also the director of the Neurosciences Institute).

Representation of the Salk Institute through Dr Cowan, is said to mark the plan that both the Neurosciences Research Program and the institute will be peripatetic, migrating *en masse* to the west coast for the summer months. Edelman hopes that the first of these summer programmes will take place this year, although formal arrangements with the Salk Institute have not yet been completed.

Polish crisis

Patriotic science

The role of science in overcoming Poland's economic difficulties will be a major point on the agenda of the next plenum of the Central Committee of the Polish United Workers' Party, Dr Hieronim Kubiak announced last week. Dr Kubiak, reputedly one of the most liberal members of the politburo, was addressing a national conference on "the role and tasks of science in getting the country out of the economic crisis".

Dr Kubiak stressed that "although science has no country, scientists do have countries", and appealed to the patriotic sentiments of all Polish scientists, asking them to rally to the new economic strategies.

From the Academy of Sciences, Dr Zdzislaw Kaczmarek, the scientific secretary, who holds quasi-ministerial rank and is accountable directly to the prime minister, announced a new government act, which will define the legal obligations implicit in scientific consultancy. Not every researcher, he said, has the qualities needed by a consultant, such as the ability to translate the language of science into the language of politics, and, most important of all, the courage to oppose "irrelevant" pressures.

The timing of the meeting was not without irony. On the same day, the Central Committee Commission for Health and Environment discussed a report on the disastrous level of environmental pollution — a subject which, until September 1980 could only be discussed in clandestine unofficial pamphlets. Even during the 16 months of Solidarity, the most revealing data, including the fluorine pollution of the Krakow area, could only be discussed in "internal" bulletins of the Polish Ecological Club. Yet now the commission cited "lack of adequate education" and information as a major cause of the current pollution levels. A few days before these meetings, another report had been under discussion in the Polish media — a discussion of the factors leading up to the imposition of martial law, produced by the "Experience and Future" group (Doświadczenie i Przyszłość, or DiP), an unofficial working party of intellectuals set up in 1978 to discuss the state of the country. In spite of official disapproval, DiP produced several reports on the economic crisis and proposed strategies for overcoming it. The latest such report, drawn up on 20 December, was ignored by the authorities until early March, when it was officially condemned as a Western forgery — although the same commentators then proceeded to vilify Stefan Bratkowski, head of DiP and chairman of the now-suspended Union of Polish Journalists. At least 30 scientists actively participated in DiP and have thus,

effectively, had their proposals for the economy already rejected. How far they are liable to respond to Kubiak's patriotic appeals is unknown.

One notable figure, however, was absent from the meeting with Kubiak — Dr Aleksander Gierynski, president of the Polish Academy of Sciences. He had been allowed to travel to Paris, to be honoured by a learned society for his contributions to historical research. The Paris ceremony was kept deliberately low-key, since Dr Gierynski had made it clear that he was present solely in his private capacity, and not as a representative of the Polish scientific establishments. **Vera Rich**

Universities in commerce

Stanford's way

Palo Alto

Graduate students at Stanford University, denied entry to this week's conference on academic-industrial relations organized by the university's president, Dr Donald T. Kennedy, are holding a rival conference of their own — in public. The Stanford graduate students' association has organized one meeting for 26 April and another for 10 May. The students' objectives are to help define their own reactions to the involvement of outside commercial interests in academic research and towards academic supervisors who acquire equity and managerial positions with companies whose interests are related to their research.

Kennedy has agreed to be the keynote speaker at the first of the students' meetings. Professor David Noble of the Massachusetts Institute of Science and Technology and author of *American by Design*, a study of the links between science and technology and the growth of corporations, will also be speaking.

For Kennedy's own meeting this week the presidents of Harvard University, the California Institute of Technology, MIT, the University of California and Stanford, together with teams of academic colleagues and industrial partners, are turning up at Pajaro Dunes. A possible code of practice to regulate the relationships between universities and commercial interests has been circulated, but even if this or some amended code is agreed on, the result will still have to be discussed within the faculties of the five universities.

One of Kennedy's messages for the students is likely to be that industrial corporations can contribute both financially and technically to the education of graduate students. He will be able to point to the arrangement now concluded between Stanford's new Center for Information Systems and seventeen major electronics companies, which will contribute \$250,000 a year for each of the next three years towards the general running costs.

Meanwhile, Stanford is taking steps to

Joint Research Centre

Jean-Albert Dinkespil, an old hand at European research efforts, is to take over from Professor Stelio Villani as Director-General of the European Community's four "Joint Research Centres". The four centres at Ispra (Italy), Geel (Belgium), Karlsruhe (West Germany) and Petten (Netherlands) employ about 2,000 European scientists, and boast a budget for the period 1980–83 of 510 million European Currency Units (£287 million).



Dinkespil, a 55-year-old Frenchman, has already seen active service at the French national centre for space studies, at the European Space Agency, as Deputy director-general of the Joint Research Centres and head of Ispra and most recently as director-general responsible for science, technology and energy at the Council of Ministers.

Jasper Becker

regularize the distribution and use of what is called "tangible research property" — unpatentable products of research which may, nevertheless, have commercial value. New guidelines for the distribution and use by others than the original scientist have been drawn up partly to draw the attention of investigators to the possible commercial importance of such things as cell lines, computer data bases and software, circuit diagrams and engineering drawings.

Dr Gerald J. Lieberman, vice-provost at Stanford, says that investigators who create tangible research property should ordinarily be the ones who decide how it should be distributed or used. Such property should not be sold for profit, but steps should be taken to see that the assistance provided by academics at Stanford is afterwards recognizable (and, if necessary, acknowledged in writing). The objective seems to be to encourage researchers to assist their colleagues elsewhere while not hazarding the university's right to control the exploitation of those developments which turn out to have commercial value.

Charlotte Beyers

CORRESPONDENCE

Creation deduced

SIR — Having recently received, for the first time, a communication from the Biblical Creation Society which contained quotations both from my letter to you of last July (*Nature* 30 July 1981, p.403) and from Dr O'Grady's comments on it (*Nature* 10 December 1981, p.510), I am reminded that a short rejoinder to the latter is needed.

It was kind of O'Grady to think that I needed information about the writings of Messrs Hume and Popper on deductive and inductive thinking. However, what I was trying to do — perhaps not clearly enough — was to put into perspective the original dichotomy between the two modes of explaining things, a division which must surely go back, as I said, to the earliest days of man as a toolmaker. In those times deduction must have more or less automatically meant a preconceived belief in, and an assertion of the truth of, dogmas and myths. Only, I suggest, with the emergence of properly understood scientific reasoning within the past few centuries, involving the *testing* of ideas, has deduction acquired its constructive aspect and become an alternative to induction in the formulation of hypotheses which make testable predictions.

My main point was that the original "prehistoric" form of deductive thinking is still with us and flourishing — perpetuating many myths both ancient and modern. I believe "creation science" is a classic example of this as it seems only concerned to propagate, in the face of a mountain of contrary evidence, a 3,000 year old myth about the origin of the Earth and of man. If this is not so, and if creationism is scientific deductive thinking, someone will have set out the creationist hypothesis in much more detail than appears in *Genesis* and will have tested it sufficiently often and successfully to think it worth continuing. Has this happened? Perhaps the three Glasgow University biochemists (see *Nature* 26 November 1981, p.302) have done this as well as simply stating, rather negatively, that the evidence does not *disprove* the existence of a Creator?

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Kindly explained

SIR — Marks¹ as well as his creationist critics²⁻⁵ seem to be arguing at cross purposes. Unlike Marks, one ought not to sneer at the views of fundamentalists, just as a clinician must not disregard the delusions of a psychotic. Both types of belief mean much to those who hold them. A creationist cannot be convinced by facts or scientific arguments of any possible errors of an *a priori* infallible creed, any more than a psychotic can be shaken about the absolute truth of his delusions by rational counter-arguments. When contrasting science and creationism we must remember that we are dealing with different *kinds* of explanations concerning the world. Let me paraphrase in an evolutionary context a passage from the late C.A. Mace written in a behaviour-scientific context in an editorial foreword to a book by Vernon⁶.

"The difference between evolutionists on the one hand and creationists on the other arises perhaps from a failure of the latter to do justice to the fact that there are many different *kinds* of explanations. There is the kind of explanation that fossils exist, which simply says that fossils are remains of past organisms, irrespective of whether these organisms were divinely created or arose by self-organization. This is a neural kind of explanation. There is the sort of explanation which appears to satisfy evolutionists and some other life scientists, which takes the form of saying that fossils are relevant to our formulations of particular theories of evolution-specific mechanisms⁷. There is another kind of explanation which satisfies materialistically-minded mechanists, who (like myself⁸) suggest that fossils are the remains of organisms that arose by self-organization. Perhaps also some notice should be taken of the kind of explanation which satisfied children and devout old ladies who would say that fossils exist because God arranged things so as to remind us of the many beautifully adapted organisms that he has created in the past."

There is room for all of these views among the right people in the right places and in mutually exclusive ways. We must remember that different kinds of explanations rely on different premises and on different explanatory procedures. Thus, hypothetico-deductive scientific theories are not intended to proclaim absolute truths, but are, or should be, falsifiable. By contrast, the doctrines of creationists are proclaimed as absolute truths of a metaphysical kind, and do not function like scientific hypotheses. If scientific facts do not fit creationist doctrine then, with sufficient ingenuity, an indefinite number of reinterpretations of the doctrinal metaphysical statements remain possible. In the Popperian sense there exists an unlimited range of built-in metaphysical defences of fundamentalist doctrines. It is therefore not uncommon for scientists to accept two schemes which may be related to the same facts. On the one hand they may accept hypothetico-deductive theories of mechanisms. On the other hand they may embrace a metaphysical belief system which explains some of the facts known to them from science in ways totally different from the ways in which they occur within scientific theories. I know many such people and some highly intelligent ones among them, although I see no personal need to supplement science with a *fundamentalist* metaphysics. (Some kind of metaphysics is probably needed in science⁹.)

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Aptly put

SIR — The comparison of spontaneous development of life to the creation of a Boeing 747 by a "tornado sweeping through a junkyard", cited by Jukes (see *Nature* 18 February, p.548) as being inept, is, on the contrary, very apt. Probably purposely, but possibly inadvertently, Jukes fails to identify the Wright brothers, Dumont and Blériot as creators. Every additional step in the development of the 747 is a result of creative minds. Without such creative genius, and with or without any number of tornadoes, a junkyard is still junk, albeit with useful materials for creative minds; and without a Creator, atoms and molecules, which He created, are still only atoms and molecules. (Go back farther to matter and energy, if you wish.) In referring to the *creators* of the modern aircraft (those he mentions and their successors), Jukes has most elegantly refuted his own argument.

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The real dangers

SIR — With regard to the correspondence from Chris Q. Doe (*Nature* 4 March, p.8), I think it is Mr Doe who is demonstrating "extreme ignorance". The statement that "any amount of radiation will produce a proportional increase in mutation rate" is an unproven supposition, and a highly doubtful one in view of recent results on the self-repairing abilities of DNA (of which Mr Doe should be aware if he is really a biologist).

As for the relative dangers of hydroelectric versus nuclear power plants, it is a *fact* that several thousands of people have been killed by collapsing dams during the past twenty years alone, while the number of fatal casualties caused by nuclear power plants can be counted on the fingers, not a single one occurring to people outside the plants (except possibly in accidents due to construction work traffic). The increased mutation rate caused by nuclear plants is, again, highly hypothetical; even if it exists, it cannot amount to more than a fraction of a per cent of the rate caused by natural radiation, itself a small fraction of the rate of mutations occurring spontaneously or caused by chemical agents. Who says that mutations are necessarily harmful, anyway? Darwin would have thought well of them had he known about them.

What science has to do with the soundness of decisions to build nuclear power plants is beyond me; as for the economics of it I leave that to the electric utilities, who presumably aren't building them just for the fun of it. Living in a country which is seeing its forests and lakes rapidly being destroyed by acid rain (already the fish are gone from 10,000 lakes in Sweden, many are practically devoid of life), I have no doubt at all, however, that nuclear power is the environmentally soundest way of producing the energy we cannot do without, and by far the one doing the least harm to nature, both momentary and permanent, and with all aspects considered.

NILS OVE BIELSTEN

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SCIENCE IN FRANCE

The grand experiment

SCIENCE in France is going for boom — or bust. The objective is to be the (socialist) Japan of Europe, and new technologies, founded on good science, will lead the way. What a contrast with the rest of the developed world. It is a great experiment, which deserves to succeed. Of course, along the way there will be many pitfalls, and in the next few pages an attempt is made to reach an objective assessment of the situation.

In fact, the experiment is not a new one. French science and technology returned to the centre of the world stage in the 1960s and 1970s thanks largely to the vision of General de Gaulle. According to figures collected by one of the present science minister's cabinet advisers, M. Pierre Papon, French science spending didn't just increase during de Gaulle's rule — it quintupled. In 1958, the French government spent FF1,540 million on research and development; and in 1967 (when de Gaulle resigned) the figure was FF8,836 million. Part of the rise can be accounted for by inflation, but even as a fraction of the government budget, the increase was staggering; from 2.5 per cent in 1958 to 6.2 per cent in 1967.

De Gaulle sought national independence, which among other things implied economic and military independence of the United States. There should be French nuclear weapons; French rockets; French electronics; French energy. And of course, French science both to support this technological development and, not least, to fly as a flag of French pride.

The experiment worked, in most essential areas (electronics still has a long way to go); and in the process science in France was transformed. There were 9,000 researchers in the state sector in 1958, and 21,000 in 1965. (The 1968 university revolution, which came the year after de Gaulle's departure, added even more: the figure was 31,000 in 1969.) This was the age of gold.

As if to confirm de Gaulle's investment, though no doubt entirely unconnected, the first of six Nobel Prizes won by Frenchmen since the war were announced in 1965: those of André Lwoff, Francois Jacob and Jacques Monod. Monod, however, gave a sour interview to the weekly newspaper *Le Nouvel Observateur* after his award, claiming that M. Georges Pompidou, de Gaulle's prime minister, was not doing enough for science. ("Pompidou never forgave him for that", says Jacob.) Perhaps it was true that more was being done for physics than biology, though one of those concerned with distributing the money in those days — M. Charles Thiebault, recently president of the Centre National de la Recherche Scientifique — says molecular and cell biology had a high priority in de Gaulle's programme. Thiebault's figures on French research productivity in the 1970s (see following page) show that the most dramatic increases in that decade came in molecular and cellular pharmacology, a fact which can be traced back, he believes, to increased support in the 1960s.

But how long can such experiments last? By the 1970s, the gold had tarnished. De Gaulle had pushed the national French research and development effort, including industry, from under one per cent of gross national product in 1958 to 2.2 per cent in 1967. Monod seems to have been right about Georges Pompidou, for when he became President the science budget began to fall. By

1980, research and development spending accounted for only 1.8 per cent of the country's gross national product. French science during the 1970s was based largely on the structure and staff given to it by de Gaulle.

Today, the unlikely inheritor of de Gaulle's scientific mantle is the left-wing socialist M. Jean-Pierre Chevènement, not as president of France, but as minister of state for research and technology with his own powerful ministry (something de Gaulle, in fact, avoided setting up). Chevènement, strongly backed by President Francois Mitterrand, is in effect relaunching Gaullism for science — albeit with a human face. The difference is that Chevènement's programmes are being designed for the people; de Gaulle's were for "France". If the difference seems subtle, that's because it is subtle. There is only a touch of democracy in Chevènement's thinking. His is a planned economy. But it is one, at least, in which the impact of technological change on the fabric of life in France will be taken into account in the development of policies.

Also, and inevitably in 1982, Chevènement's choice of *grands programmes* differs from de Gaulle's. The new minister does not control defence research (which was 65 per cent of de Gaulle's first research and development budget). So physical science will not be so prominent. In fact, the most advanced and active of Chevènement's plans concern biotechnology, with its spin-off of support for molecular biology, microbiology and enzymology.

The 64 million franc question, however, is whether the plan will work at all. The whole economic exercise now under way in France is a gamble on investment: the exact opposite, say, of British policy. The projected budget deficit of the French government for 1983 is now FF 200,000 million (£20,000 million). Moreover there are structural and psychological obstacles to the further development of science — and in particular, technology — in France. These centre around the strong, historic distinction that has been drawn in French higher education between training (represented by the *grandes écoles*) and culture (represented by the universities). De Gaulle probably went as far as he could without changing this system substantially. Chevènement, needing to go beyond de Gaulle's achievements, may not have that luxury. □

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Has France got the money . . .

It seems that France is now committed to an immense and breathtaking economic gamble, like a hardened punter throwing all his chips on one spin of the wheel. In direct contrast to the straitlaced grocer-shop monetarism now the fashion in Britain and the United States, the French solution to the recession, to unemployment and the challenge of Japan is to spend, spend, spend.

Consider the ministry of industry. Capital aid to industry and business — excluding the defence industry and support for research and development — has grown 80 per cent this year to FF8,000 million (£800 million). Against such massive increases in direct support to industry, the 29 per cent increase in the government research budget for 1982 looks almost niggardly. Moreover, the increase in spending money — as opposed to commitments to order, but not actually buy, equipment and materials — has risen only 23 per cent, or 9 per cent allowing for current inflation; and since the US dollar has risen 50 per cent against the franc in the past two years, and many materials and much equipment must be bought at dollar prices, some laboratories claim that they are at best maintaining their real level of expenditure. Furthermore, some 25 per cent of this year's new money may be with-

held if the economy does not perform as hoped, so there are growing doubts among researchers as to the true value of the "boom".

Nevertheless, Jean-Pierre Chevènement, minister for science and technology, is giving major boosts to certain areas, which must survive the worst of disasters. ANVAR, the Agence Nationale de Valorisation de la Recherche, which seeks to convert research in university and government laboratories into productive industrial innovations, will increase its budget by 76 per cent this year; alternative energy research grows 50 per cent; and aid for the dissemination of scientific information, up 80 per cent.

Meanwhile the budget and foreign trade deficits balloon massively. The total government budget will increase by 28 per cent this year — leaving an estimated deficit of FF95,000 million (£9,500 million). The foreign trade balance (dominated by oil imports) is expected to be in deficit to about FF60,000 million (£6,000 million). For rough comparison, Britain's current account deficit is about 10 times less severe, when oil revenues are subtracted. (Including oil revenues, Britain is in the black to about the same degree that France is in the red.) around: the Mitterrand gamble is a long one.

Read right on from here

IN the following articles, there is an Anglo-Saxon look at France in something approaching a logical sequence. But it may not be logical enough for a French man or woman. Logic is a great French strength, but also a weakness. The greatest respect goes to the most abstract disciplines: purest of pure mathematics, the most theoretical anthropology. The schools create this love of logic, and the *grandes écoles* foster it.

Logic is also linear, and the French certainly love the direct route. Cross-fertilization and mobility seem to be foreign ideas. The author of a recent report recommending the establishment of an agency for technology assessment wrote "in France, there are traditional difficulties in thinking in terms of complex systems and networks . . ."

Careers also are seen linearly. A vogue word these days is *filière*, which is usually applied to industry: a *filière* is a whole chain of connections which takes a primary material to its marketable product. There are *filière électronique*, *filière énergétique*, and *filière what-you-will*. Industry is a clutch of distinct *filières*. It is the same with careers. One follows one's chosen career, with a series of qualifications marking progress and serving to attach one firmly to this line rather than another.

In the following pages, some of these threads are laid out: the line of the *grandes écoles*, which leads to industry and the ministries, and skims off many of the most able pupils; the line of the universities, still wavering after the "revolutions" of 1968, where most French research is done; and the line of the *grands organismes*, the big state institutions which fund and control the best of that research.

The new politics of research are outlined on page 299 — the minister for science, Jean-Pierre Chevènement, wishes to push French research and development spending up by 25 per cent in real terms by 1985, to 2.5 per cent of the gross national product. Chevènement may be spending more money and employing more people, but he is also attempting to link a few of the stubbornly separated *filières*: to link industry with the *grands organismes*, research with the *grandes écoles*, thereby seeking greater productivity from French science.

At the same time, Chevènement will be creating some *filières* of his own: a number of full-scale national research and development programmes, after the style of de Gaulle before him. We sketch one: biotechnology (page 295). Finally, we review two of the most successful existing French technological programmes: space and nuclear energy. □

. . . and the science?

FRENCH science is on the move — upwards — according to figures prepared by the ex-president of the Centre National de la Recherche Scientifique, Professor Charles Thiebaut, with help from CNRS and the Pascal bibliographic data bank (see table).

Thiebaut calculated the percentage of research papers of French (and other non-US) origin in the 286 most-cited journals of *Science Citation Index*, and compared two years as near as possible to 1970 and 1980. By this measure, all French science except physics increased in literature penetration during the 'seventies. The best single penetration at the end of the decade was achieved by French astronomy: in 1979, 10.8 per cent of the surveyed astronomy papers were French. The best improvement was in pharmacology, from 0.5 to 5.5 per cent. Most of the papers were in English.

By comparison, the United Kingdom suffered a dramatic decline.

As with all such figures, however, the data must be interpreted with caution. The number of French scientists leapt up during the expansion of the universities in 1968–71, so to some extent increased penetration is a measure of increased quantity rather than quality. For example, French mathematics is certainly far better than seems to be indicated by its penetration of the literature. Since 1945, the Fields medal for mathematics — the

"Nobel Prize" of the subject — has gone five times to France, compared with only eight to the United States (which has five times the population). By contrast, in the scientific Nobel Prizes, France has been weak: six since 1945 (two in physics, four in physiology and medicine) compared with 13 in Germany and 35 in Britain.

Fraction of papers (%) in major scientific journals

Disciplines	West				
	France	Germany	UK	Japan	Canada
Mathematics					
1970	3.4	9.0	10.0	1.0	4.5
1980	7.2	11.5	6.9	3.3	3.4
Physics					
1973	8.5	6.4	10.2	4.0	4.5
1979	8.2	8.4	6.7	5.3	4.3
Engineering					
1972	4.5	4.7	15.5	4.6	5.6
1979	5.9	5.5	8.8	8.1	4.2
Chemistry					
1970	2.9	3.7	16.2	5.9	4.2
1980	7.3	5.8	10.5	8.8	4.5
Geophysics/Space					
1972	3.5	3.1	10.4	1.9	7.1
1979	5.0	3.7	9.3	2.4	6.6
Biology					
1970–73	2.3	4.6	16.0	2.8	3.9
1979–80	4.4	5.0	12.0	4.7	4.6

Thiebaut himself interprets the figures as a good broad measure of the productivity of the community of French scientists, indicating that France is now producing science "at the normal rate". But he is worried about what will happen in the late 1980s. Scientists are most productive in their 30s and 40s, Thiebaut believes, and the drastic fall in French recruitment of young scientists in the 1970s may ultimately show through in output.

Great schools, great contradictions

THE most extraordinary thing about the French education system is that it is dominated by science, and yet it is inefficient at producing scientists. At the age of 14, children are selected to study for this or that branch of the *baccalauréat*, or "bac". The most able are groomed for bac C: mathematics and physical science. To do anything else is, by comparison, to opt out of the academic race. Bac C pupils are virtually the only entrants to the *grandes écoles*, the higher education production lines of the French élite. When bac C pupils go to university, as nearly half of them do, they do better than any other class.

Yet of this leading group, only a minority choose to study science. Have they had enough of it? Roughly one in eight go on to short-term technology courses at "instituts universitaires de technologie". Another one in eight (about 3,000 a year) study science at university. Two-thirds of those who go to university study something other than science.

The contradiction goes deeper. The really ambitious bac C pupil goes on to two-year preparatory classes, cramming schools aimed at the special entrance exam to the *grandes écoles*, the *concours*. The preparatory courses are called *taupe* (and *hypotaupe*) — that is, "mole" and "hypomole" — for good reason. The work is so intense that the student never lifts his or her head to the light of day. Once a student is successful in the *concours*, the *grandes écoles* themselves are a relaxation: a qualification and a good job are almost guaranteed.

What then do these moles learn when they emerge into the *grandes écoles*? Not "science", properly speaking. The teachers, on the whole, do not do research. There are great variations in coverage, and in quality, but what is called "engineering" dominates. Of 300 or so *grandes écoles*, 160 are engineering schools. Another 60 are business schools, and another 80 cover miscellaneous arts and occupations. Two or three schools do have substantial research departments, including the prestigious Ecole Normale Supérieure, and so teach science effectively — but only to a few hundred, in total, of the bac C élite. Moreover the science is almost exclusively physical; biology has until recently been largely neglected.

Even the engineering taught by the *grandes écoles* is said to be too theoretical, too dry and too dated to be good engineering (though there are important exceptions, of course). So what do the *grandes écoles* teach their students? They teach rigour in argument, and — a practical matter — how to take decisions.

The subjects studied might as well be classics for all the significance they have. The jobs pages of *Le Monde*, the principal French newspaper, are plastered with adverts for *ingénieurs grandes écoles* not

because these are engineers, but because these will be the most intelligent managers. The ministries of the French state also recruit most of their top staff from the *grandes écoles*.

The remarkable result is that while French education stresses science from the age of 14, the product is a group of managers and civil servants who could hardly be further from research. The university system, where research is done, is seen very much as second class, and the social distinction is maintained throughout life. The club of the *grandes écoles* is exclusive, and the damaging consequence is that research penetrates industry far less than it should.

The universities themselves have their own problems. They were not ready for the student population bulge of the sixties, and together with certain social and political factors the result was the transformation of 1968, whose impact has still not been properly assimilated.

The rise in student numbers was dramatic. In 1960, there were 223,000 students studying in the French universities — which were then arranged as almost totally independent, unconnected faculties specializing in this or that discipline. By



(Plantu, Le Monde)

1968, there were more than 575,000 students cramming into the same lecture halls. "Often there were hundreds at a lecture", wrote one observer, "many of them sitting on the floor right up to the blackboard. Students had to arrive early to get a place". The chalky lectures seemed out of touch with the concerns of youth: the Vietnam war, alternatives to "scientism" and economic growth. The students demanded change, and got it.

The present French universities, apart from ancient establishments like the University of Paris, are largely the creations of 1968. Faculties were grouped into multidisciplinary campuses. Students were given rights in university administration, new courses started, new



First lady at the École Polytechnique: Anne Chopinet graduates in 1972, one of a group of seven who were the first women ever to study there. Now she deals with mineral resources and uranium exploration at the ministry of industry.

universities founded, and new lecturers recruited. Recruitment had been going on earlier, however. In 1960, there were 10,000 university teaching staff from professors through *maîtres de conférence*, *maîtres assistants*, to assistants. In 1967-68, the number had reached 26,000. Some 2,000 assistants were recruited that year. But in 1968-69, the number of appointments was 4,500; in 1969-70, 2,500; and in 1970-71 (slowing down substantially) 800. By 1973-4 the number of teaching staff was nearly 41,000 (where it has roughly remained for ten years). Thus in 1980 there were 41,000 lecturers and 861,000 students, a university system almost exactly four times that of twenty years before, containing a mass of assistants created in one brief five-year period. Throughout this higher education boom, the *grandes écoles* remained aloof, taking almost constant numbers.

The suddenness of this growth, and the continuing strong position of the *grandes écoles*, has meant that the universities have found themselves almost entirely detached from the centres of power in France, from industry and from government (which nevertheless keeps them on a tight rein, like an errant child). There has thus grown up a feeling — in part a legacy of 1968 — that the university and its research should not be sullied by contacts with industry. This has become a principle, one which also gives the new university system a feeling of dignity in French life.

The distinction is even reinforced by law: university staff are civil servants who, in principle, should not seek consultancies in industry; and the universities should not enter into any kind of profitable liaison with a company. Needless to say, the more enterprising universities and staff have set up such links by devious means; but the law sets up obstacles. (One of the most important exercises undertaken by the new

minister of science, Jean-Pierre Chevènement, therefore, will be to remove these legal trip-wires, and the forthcoming "law of research" (see page 289) should do so. But the established suspicion of industry will remain.)

There is also another weakness in the French university system: its relation to research itself. The control and management of French science rests essentially with the *grands organismes*, roughly equivalent to other countries' research councils, but more powerful and paying more salaries. The *grands organismes* draw their researchers from the university system, give them better research facilities, remove their heavy load of teaching and administration but — for the moment — give them worse conditions of employment: lower pay and fewer rights, even though they may be working in the same campus, even the same laboratory as university "teacher-researchers".

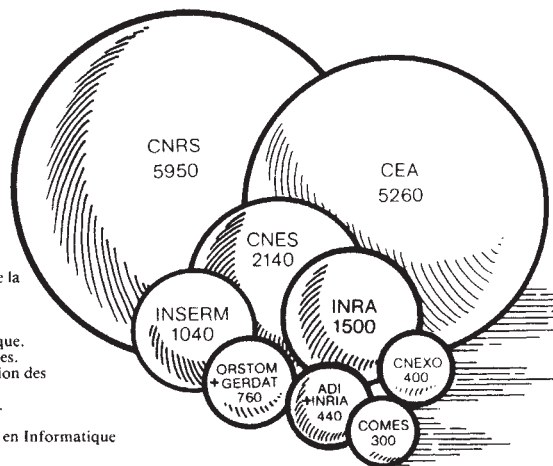
Most scientists, however, are keen to make the deal — as is evidenced by the nearly eight-to-one ratio of applicants to posts at the junior *grands organismes* level of *attache* (see page 287 for details of the more prominent *grands organismes*). The *grands organismes*, plus one or two separate research institutions like the Institut Pasteur (biology) and the Institut des Hautes Etudes Scientifiques (mathematics and theoretical physics) are the heart of science in France.

Now comes the crunch: since *grands organismes* researchers tend not to teach,

France's big spending research organizations

The "Grands organismes de recherche" whose budgets come under the wing of the ministry of research and technology. The 1982 budgets are shown in millions of francs.

ORSTOM:	Office de la Recherche Scientifique et Technique — Outre-Mer.
GERDAT:	Groupe d'Etudes et de Recherches pour le Développement de l'Agronomie Tropicale.
CNRS:	Centre National de la Recherche Scientifique.
INSERM:	Institut National de la Santé et de la Recherche Médicale.
INRA:	Institut National de la Recherche Agronomique.
CEA:	Commissariat à l'Energie Atomique.
CNRS:	Centre National d'Etudes Spatiales.
CNEXO:	Centre National pour l'Exploitation des Océans.
COMES:	Commissariat à l'Energie Solaire.
ADI:	Agence de l'Informatique.
INRIA:	Institut National de la Recherche en Informatique et en Automatique.



and since they do the best research, higher education is denuded of its leading scientists. So how are scientists trained?

This may become one of the most important issues in French science policy, for with the promised civil servant status for *grands organismes* researchers, matching that for university teachers, there will be nothing to keep researchers in the lecture theatres but a dedication to teaching. Much is now being made of the catch-phrase "education for and through research", but the means are still being sought to bring it to reality. The *grands organismes* are now under pressure to play a greater role in education, and they must

take it up if they are to find the scientists they need in future years — but the universities are very wary of such moves, seeing them as an encroachment on the territory.

All in all, there is a lot to be done. The government does seem to be aware of the problem: the minister of education, M. Alain Savary, promises a new law for the universities by the autumn, and he is working in close collaboration with his pushy opposite number in the ministry of research, Jean-Pierre Chevènement. We shall see then if anything substantial and long-lasting is to be achieved with French science. □

What Aigrain thinks of it all

THE man who must look most wryly at the present efforts to create a renaissance in French science and technology is Pierre Aigrain, now director of research and development at Thomson CSF, but minister of science under Giscard d'Estaing.

Wryly, because he had begun to push up the science budget in 1981 (not so much as Chevènement in 1982, but the latter's efforts are carried on the tide of an overall 27.6 per cent increase in government spending). Wryly, because the politics of opening science to industry were his own. In some areas Chevènement has even taken on the very advisers that Aigrain used. Chevènement appears to be getting the glory for a policy which, in large part, was established by Aigrain. So what does he think of his successor's plans?

Aigrain is "very glad" about the proposal for a law that would more or less guarantee the science budget for a few years (see opposite). But otherwise he feels there has been a change of wording more than a change of substance. Aigrain clearly thinks that the National Colloquium on science and technology, which drew together scientists and others influenced by their work in a great

jamboree of meetings late in 1981 and early this year, was a marvellous idea, for the real problem of development in France is a matter of psychology as much as one of regulations and budgets; the colloquium demolished some barriers.

However, he is worried about Chevènement's accelerations of scientific recruitment to the *grands organismes*



Pierre Aigrain — pleased with the "loi programme" but worried about industry

(like CNRS) from 3 per cent of staff per year (under his ministry) to 4.5 per cent. There are enough good people to fill the quota, says Aigrain, but not enough left over to satisfy the needs of science-based industry; and since the security and terms of employment in the *grands organismes* are excellent, industry is going to have a

hard time reaching the 8 per cent a year real growth in research and development demanded by the new minister.

Education needs an overhaul, and the government is aware of the problem, Aigrain believes, but an education system has a lot of inertia. Moreover, the source of the problem lies in the secondary schools, so change effected there will take ten years to have an impact on research.

The secondary schools overstress the mathematical and physical sciences, are too abstract, and demand too much of their pupils, he says, "so people who would have been quite good physicists and electrical engineers turn to biology to avoid the 'terrorist' attitude of our mathematicians". The result is too many biologists and far too many social scientists.

The *grandes écoles*, the majority of which teach engineering, also have a lot of drawbacks. They are rather small, and so produce too few engineers. On the other hand, the universities have completely neglected to train people for industry, and although good research is done, little of it is relevant to industrial problems. "What should be done is to improve the university system and its links to industry, and the selection of university students", Aigrain concludes.

Judy Redfearn & Robert Walgate

No union dominance at the CNRS

The minister answers some questions

THE new politics of research and development in France raises many questions, but some are particularly troublesome. How far will Jean-Pierre Chevenement, research and technology minister, go along with trade union demands for democracy in the laboratory? Why does he insist on the importance of French as a scientific language? And finally, what can he do about getting more scientists into industry?

The first question is particularly topical, for the scientific trade unions in the Centre National de la Recherche Scientifique (CNRS), the leading basic research institution in France, have buried their differences to demand union, and only union, representation on the *Comité National* — the all-important “parliament” of CNRS (see “Pressing problems for Payan”, page 293). What does Chevenement think of that Robert Walgate asked him?

The minister says he is at pains to preserve the “effectiveness and openness” of the *Comité National*, which must play a greater role than before in determining the policies of the CNRS. But he added that although there would be “no question” of giving a monopoly of seats to the unions, the seats should be open to the unions (through election). It would also be a good thing, says Chevenement, if the unions began to concern themselves more with “the quality of research” in their politics, a remark that indicates that he would like the union to broaden their horizon. Nevertheless, says the minister, a union presence “raises the level of debate”.

As for promoting the use of French in science, Chevenement says the objective is to help communicate science to the public, and to those who might make use of it in industry, “to maintain the strict link between the French scientific community, and the national community, which would be put under great strain if French ceased to be the language of scientists . . . French must be defended even in areas of research where the rule is to use English.”

It follows that all French research must be published “at least” in French and, “if possible”, in other languages, says the minister. In conferences held on French soil, French must be one of the working languages. A long-term programme must be undertaken to “renew French scientific and technical publications”. A government supported publishing house will be set up for this purpose, says Chevenement and there will be “a vast effort” to translate foreign publications into French.

Many have claimed that the cost of such

a programme will make it impractical, but “it will not cost as much as they say” says the minister. The budget for the programme this year is FF 70 million (£7 million). But Chevenement adds that this budget is planned to grow “considerably” in the years to come.

Finally, research in industry will be supported by plans to train 1,500 engineers a year “through research” by 1985, compared to only 500 a year at present. (France produces some 11,000 qualified

engineers a year at the moment, but only a small fraction have training in research.) Particular efforts will be made to train the engineers and scientists needed for the *programmes mobilisateurs* — the special long-term programmes of the ministry, such as biotechnology and electronics. “This is in line with a major policy of training through research that we think will resolve the problems of high-level scientific recruitment, both for the public research organizations and for industry”. □



“Make France the third scientific and technological power of the world” François Mitterrand (right), President of France, has told minister of state for research and technology, Jean-Pierre Chevenement (left).

A new law for science

ONE law for the military, one for science: the management of the defence budget was foremost in the science minister’s mind when he created a “programme law” for science which would define the budget and orientation of his ministry for five years. The ministry of defence employs a series of such laws, which avoid the frequent battles for funds and policies.

The result is the “*loi de programmation et d’orientation*”, now before the French economic and social committee for comment and possible amendment. The law fixes a government civil research and development budget increase of 17.8 per cent a year in real terms, averaged over the three years covered by the law. Fundamental research gets a 13 per cent per year increase. Jobs in science will be created at the rate of 4.5 per cent of existing positions a year (on average). And support will be given to industry to increase research budgets by 8 per cent a year.

No details are given in the law of how the money is to be spent, which has been taken by some to be a weakness; on the other hand, the ministry sees this lack of detail as a strength, giving it room for manoeuvre.

Moreover, one major financial battle has already been won: in the section on *grands organismes* the law speaks of financial control *a posteriori*. Up to now the ministry of finance has held very tight rein on the details of budgets with laboratory directors consequently unable to make even minor changes in spending

once the budget for a year has been agreed. Under the new law, there should be more freedom.

This most important section of the law also redefines the role of the *grands organismes*. They would now have four tasks: to pursue research in every domain; to ensure discoveries are brought to application wherever possible; to spread knowledge; and to educate and train people “through and for” research. The law would also give the *grands organismes* a better legal framework in which to cooperate with industry. This would allow CNRS, for example, to join with and even hold shares in sister companies.

Researchers in the *grands organismes*, however suspicious they might be of industry should welcome the provisions in the law for a new contract of employment which would give them something approaching civil servant status.

Other important provisions in the law are an emphasis on regionalism and an identification of the main programmes of research to be developed by the ministry. These are: the rational use of energy and new sources; biotechnology; electronics; research in cooperation with and for the Third World; research on working conditions and the impact of new technologies; the promotion of French as a language of science; and technologies related to French industrial development not included in the above (with the exception of nuclear engineering). □

Science — the next revolution?

THE Mitterrand government wants to make its mark on history. So for what will we remember the minister of state for research and technology, Jean-Pierre Chevènement?

Quite likely for what the newspapers dubbed the "States General" of science, an apparently formless meeting in January this year of some 3,000 French men and women representing almost every nook and corner of French science and technology. This National Colloquium on Science and Technology was merely the culmination of a large series of regional meetings — the *Assises Régionales* — which took place the previous October and November, and generated 200,000 pages of detailed, local, reports and criticism on the management of science in France.

Why "States General"? *Les États généraux* were a feudal institution, last used by Louis XVI in 1788, in which representatives of all the parishes of France were called to Court to make their concerns known to the king. Louis himself misjudged them. The States General of 1788 raised such hopes that they led directly to the French Revolution of 1789.

So the National Colloquium of 1982 was a "States General" of science. That's not to suggest that next year there will be a revolution — but great hopes and fears have been raised among the scientific masses, and Chevènement now has to ride this flood or be submerged. Already certain trade unions, strong in French science, are threatening a struggle (see below). Whatever happens to Chevènement, he will be remembered for having created the National Colloquium. Pierre Aigrain, Chevènement's predecessor as

minister of science, clearly regrets that he did not think of the idea himself. . . .

So what are the main currents at work? It's not possible here to do justice to the 200,000 pages which are the main force of the colloquium. But here are a few individual views.

Take Professor Louis Lliboutry, for example, a world-renowned glaciologist with a position at the University of Grenoble and a laboratory supported by the Centre National de la Recherche Scientifique, (the major basic science organization in France). His subject is not one of the current fashions; glaciology does not have much impact on technology and the French economy; the laboratory is away from the centre of power in Paris.

Yet Grenoble is an excellent place to do glaciology, says Lliboutry — he can be at Mont Blanc in an hour by car — but he felt this subject had been neglected by CNRS. "Our budget increases have not kept up with the cost of living", he said: and while the real money had shrunk, CNRS had shifted more and more resources out of the general budget and into special programmes, the Actions Thématiques Programmées (ATP). So in the past if your research did not fall within the framework of an ATP, you were neglected. In fact the exercise of raising cash had become largely one of convincing the relevant committee that your research fell within this or that ATP. For glaciology, one could sometimes make contact with the physics of tectonic plate movement or climatology, for example. "The people in the committees are the most noisy, but not necessarily the most brilliant", said Lliboutry.

There is an alternative now, however, on

the ATPs — whose use increased greatly under Giscard d'Estaing's science minister, Pierre Aigrain. That's that they provide an opportunity for new researchers and new groups to draw on CNRS funds, in competition with established laboratories.

Unfortunately for Lliboutry and other laboratory directors who share his views, the emphasis on ATP increases slightly under the new government: CNRS funds for ATP rise 41.5 per cent this year compared with last. General programme support in CNRS will also rise, but less strongly: 36.8 per cent. Funds for big equipment (costing more than FF 100,000–£10,000) increase least: 10.5 per cent.

However, another of Lliboutry's worries may be dealt with by the research "law", the "*Loi de programmation et d'orientation*" (see page 289) which is currently receiving the attention of the economic and social committee of the French Assembly. The problem is flexibility in the use of funds.

"We lab directors have no free money" says Lliboutry. This is the real obstacle to good research — what Lliboutry calls "the Kafkaesque rules for finance". These are imposed by the Ministry of Finance, but also by CNRS itself. For example, suppose Lliboutry asked for FF 100,000 for glaciology equipment and another FF 100,000 for general research expenses, for a project within some ATP; and suppose that ATP committee awarded only the latter FF 100,000 but ignored the equipment request. Then Lliboutry could not divide the sum 50:50 as he might wish.

This might change — at least in so far as the Ministry of Finance is concerned — if the *loi* is passed by the assembly, for the law will loosen constraints of this kind on CNRS. How far CNRS will choose to devolve this new freedom to its laboratory directors, and how far it will want to keep control through its central committees, is undecided, however.

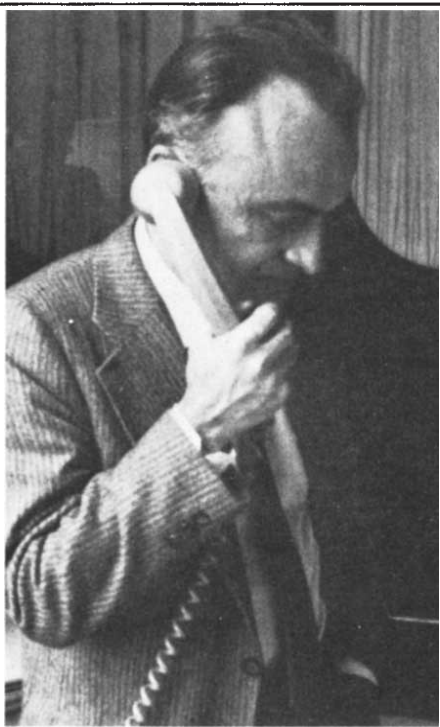
Jean-Jacques Payan, the recently appointed director-general of CNRS, would in fact be well-advised to devolve this new power (if, of course, the Assembly approves it). Lliboutry's complaints are far from unique; they represent a widely-felt unease in the regions of France that the control of science has always been too hierarchical, too Paris-based, and that the minister for science, Jean-Pierre Chevènement, while paying lip service to "democracy", has in fact such well-formed plans that Paris control will continue as strongly as before.

"We need a local *esprit d'entreprise* in small science, with the freedom and the power to make judgments locally", Lliboutry suggests, with decisions up to a certain level taken by local laboratory councils. Many speeches and papers at the *Assises Régionales* and the National Colloquium made the same or similar points. The *loi*, it seems, will set up regional science committees charged with esta-

Colloquium into law

PIERRE PAPON (right), professor of physics in the University of Paris, is a prominent member of the science minister's cabinet. Papon was responsible for assessing the sometimes diffuse results of the National Colloquium on Science and Technology, and putting these conclusions into effect in the new law for science (see page 289).

Papon identifies four principal effects of the colloquium on the law: on regional development (regional councils for science were proposed at the colloquium, and have found their way into the law); the principle of education through and by research — which is to say that the *grands organismes* should have a role in education as well as the universities; other improvements in the training of scientists; and the recognition of research as a career so a researcher could expect similar conditions of employment in different organizations, to allow more mobility.





The CEA research centre at Grenoble, on the borders of the French Alps. The dome of the Institut Laue Langevin reactor building can also be seen on the left. The area includes many important laboratories and represents the largest concentration of physical science in France outside the Paris region.

blishing regional science policies and spending a certain amount of money, but it is unlikely that these bodies will have much effect on basic science.

The opposite concern — expressed at the other end of affairs, in Paris — is that there is a degree of rigidity in the system of science management, caused in part by the existence of laboratories whose directions are difficult to shift. Although in principle research posts with the big three research organizations — CNRS, INSERM (for medical science), and INRA (for agriculture) — are contractual, and assessments of performance of staff at laboratories are made regularly, laboratories are very rarely closed down and people almost never fired. Professor Charles Thiebault, who resigned last year as president of CNRS over a conflict with science minister Chevènement, says that of the 1,200 or so CNRS laboratories (one third of all university laboratories), perhaps one or two were closed each year and staff were moved on to new work. Moreover, a new contract for all research workers, contained in the *loi*, will give researchers even more security, matching that of civil servants.

Professor Michel Petit, director of INAG, which is effectively a wing of CNRS concerned with managing astronomy and geophysics, points to another stiffness in the joints: applicants for CNRS posts specify which laboratory they wish to join. So although assessment is made nationally, keeping standards up, students tend to return to their old professor's laboratory, reducing mobility.

These are directors' complaints, however. Down at the grass-roots, some rather more anarchic notes are being struck. At the Strasbourg Assize Regionale, for example, there was a strong call for laboratory directors' posts to be made fixed term — perhaps four years — to stop one man gaining lifetime control over a sector of research, and allow a few new

flowers to bloom occasionally.

Even at the Institut Pasteur in Paris, a world-renowned centre for microbiology and molecular genetics, there is a degree of revolt. Laboratory directors should be continuously assessed, some of the younger researchers say. There is a tendency for group leaders and laboratory directors in France to stop doing research and become full-time politicians, they complain — quite unlike the United States, where group leaders might spend time rooting for money but do not neglect the life of their groups. Jacques Monod, the famous director of the Pasteur in the late 1960s and early 1970s, is said to have been an exception. Certainly François Gros, the previous director of the Pasteur, has taken the political path: he is now science adviser to the Prime Minister, Pierre Mauroy.

The myth of the "*savant*", the wise man to whom all possible respect is due, is also too strong in France for the health of science, young researchers say. Strangely for the country which once slaughtered its aristocracy, there is an almost servile respect for these intellectual aristocrats.

According to Professor Bernard Jacrot — himself a potential *savant*, who directs the neutron scattering outstation of the European Molecular Biology Laboratory at the Institut Laue Langevin, Grenoble — the problem starts in the secondary schools. "As soon as a pupil tries to be individual, he is stamped on" he says; and this reflects itself in the structure of the French laboratory, which is very hierarchical "leaving little room for originality".

Thus there is a need to give younger researchers their head, perhaps against the will of the all-imposing director, and this is a theme which the powerful French scientific trade unions have taken up with enthusiasm. But there is a difference between flattening the pyramid a little, and turning it upside down.

However, one of the clearest democratic

Big physics

THE Commissariat à l'Energie Atomique (CEA) has a civil research budget for 1982 of FF5,260 million, exceeded only by that of the Centre National de la Recherche Scientifique. The budget is high not so much because the CEA supports a lot of researchers (nearly 9,000 at CNRS, only 900 in fundamental research at CEA), but because the bulk of CEA research is in the applications and development of nuclear energy. The fundamental science is also expensive — reactor physics, accelerator physics, nuclear fusion and high energy astronomy. However, CEA researchers also work in condensed matter and materials science and chemistry and biology (using isotopes). The fundamental research budget of the CEA in 1982 is FF 1,292 million.

The CEA works in strict liaison with the other *grands organismes* on many joint programmes (such as the nearly-completed nuclear accelerator GANIL, in conjunction with IN₂P₃) and with international organizations such as the European Centre for Nuclear Research (CERN) and the Institut Laue Langevin. Its principal research centres are at Saclay, Caen, Fontenay-aux-Roses, Grenoble, and Cadarache.

indications of the wishes of basic scientists has come from an internal survey conducted by CNRS. Professor Guy Aubert, director of the Service National des Champs Intenses, was charged with collecting the contributions of CNRS staff to Assizes Regionales and related meeting throughout France. Five main themes emerged, said Aubert.

First, people were worried that Mitterrand's and Chevènement's commitment to regionalism would mean that peer assessments and appointments would be made and decided regionally. This was thought to be a bad thing, because a critical mass of assessors could not be reached and local savants might become even more influential.

Second, there was strong pressure for more "democracy" in the laboratory — the banner which the unions are flying. There should certainly be consultation through a laboratory council, including administrators and technicians, over major developments. And there should be elections "even, and perhaps especially, of the laboratory director" (Aubert sums up).

Third, it was felt that there was more room for research in the social sciences — not just more money for the field, but a re-orientation of priorities towards current political, economic, and social concerns in France. This view is certainly shared by Chevènement, as the resignation of Charles Thiebault (mentioned above) as CNRS president was the result of the

minister's efforts to force a social scientist of exactly this persuasion onto CNRS, as social sciences director.

Fourth, there was much puzzlement over the role and relationship of all the big organizations which control and fund science in France. "It seems to people in the laboratories that these things are not clear" says Aubert. It is possible to obtain money for a given subject from many different sources, under different rules, and career requirements under different heads for essentially the same work can be quite distinct. This leads to a confusion of careers and priorities, and a reinforcement of the feeling that policy is decided behind closed doors according to unknown rules. Chavenement has said he seeks "transparency".

The situation seems to be most extreme for the medical sciences, and basic sciences bordering on medicine — going as far as molecular biology. Here there are the CNRS, whose major sector is life sciences; INSERM, the Institut National de la Santé et de la Recherche Médicale; the Mission de la Recherche of the Ministry of Universities; the universities themselves; and the teaching hospitals. Dr Jean Dausset, for example, is at the Hôpital St Louis in north Paris. He is also head of an

INSERM unit. And co director of a CNRS laboratory. Or take Professor Pierre Chambon. He is a professor at the Université Louis Pasteur in Strasbourg, and head of an INSERM unit and director of a CNRS laboratory, under the same roof. It is good to know that the new director of INSERM, Professor Philippe Lazar, and the director of life sciences at CNRS, Professor Roger Monnier, aim to resolve the issue.

The fifth and final concern detected by Aubert's review of CNRS papers to the colloquium and its tributaries was over the matter of contracts. As one foreign observer and francophile put it, the urge for security is extreme in France (so the trauma of two million unemployed can be imagined). So unions have been pressing, essentially, for tenure, and a strict demarcation of jobs; conditions of work like those of civil servants (the goal, it seems, of every Frenchman and woman); and an "open" assessment of researchers.

Some of these demands the unions will achieve in the "loi"; others they will not. But the unions are pressing harder on this left-wing government than they did on the previous right wing one, much to the socialists' embarrassment; and what the result will be is anyone's guess. □

strengthen existing ones.

It is perhaps unfair to make selections, but some of the outstanding regional centres are Grenoble and Lyons (for physics), Strasbourg (molecular genetics), Toulouse (biotechnology and space science) and Marseilles (immunology). The unique technological University of Compiègne — it can probably be counted just outside the Paris region — is also outstanding in enzymology, particularly as it relates to biotechnology.

The largest sector of the CNRS budget for 1982 goes to life sciences (25.5 per cent), which looms larger than it might because some of the heavier expenses of big science are borne by or shared with the Commissariat à l'Energie Atomique (for nuclear physics) or the Centre National d'Etudes Spatiales (for satellite-borne astronomy). Even so, life sciences are also supported by the Institut National de la Santé et de la Recherche Médicale — whose budget exceeds that of the life sciences sector of CNRS though employing fewer researchers.

Other sectors of CNRS are chemistry (15.7 per cent of the 1982 budget), nuclear and particle physics (12.9 per cent), earth, atmospheric, ocean and space sciences (11.8 per cent), mathematics and basic physics (11.7 per cent), social sciences (8 per cent), physical sciences for engineering (7.9 per cent), humanities (5.5 per cent), and interdisciplinary programmes (1 per cent).

CNRS is in fact organized as a "group" of three bodies: CNRS proper, plus INAG and IN₂P₃. The latter are the Institut Nationale d'Astronomie et de Géophysique, and the Institut National de Physique Nucléaire et de Physique des Particules, and they manage the parts of the total CNRS budget concerned with those fields. INAG and IN₂P₃ are loosely controlled by the CNRS directorate, which selects the INAG and IN₂P₃ directors (from lists of "at least two" names proposed by these bodies) and channels funds to the two

CNRS — the core of research

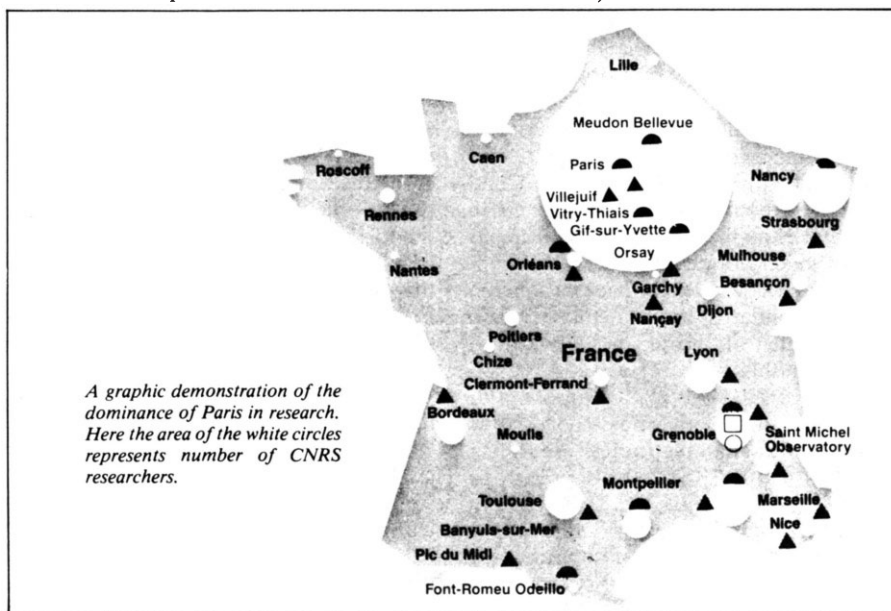
THE Centre Nationale de la Recherche Scientifique (CNRS) has FF 6,000 million to spend this year — about £600 million, including salaries. CNRS attracts and controls the cream of most basic science in France — with the exception of mathematics, which has a very strong French historical tradition and leads a more independent life. With that exception, the goal of most aspiring basic scientists in France is to join a CNRS group or laboratory — a goal which has become even more attractive now, since the new government has increased scientific recruitment to CNRS and its junior partners by 50 per cent, and now that CNRS scientists are promised essentially civil service security, pensions and other rights.

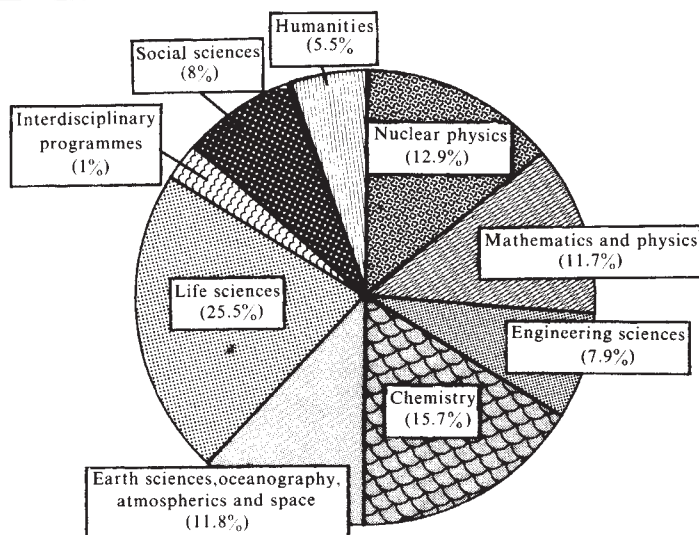
Entry to CNRS is by examination and interview. In 1980, for example, around 3,000 young scientists applied to join CNRS. Most of the 3,000 had just completed a *troisième cycle* (something between a master's and a PhD degree) at a university; 425 of them were selected as *attaches* on a four-year trial, after which, according to the 3 per cent recruitment then in force, some two out of three might have expected to receive a full-time appointment as *charge de recherche*. With 4.5 per cent recruitment, possibly all of this bunch will be lucky.

CNRS now employs 23,320 people, of whom 8,970 are researchers, distributed in more than 1,200 laboratories. More than 150 laboratories are fully controlled by

CNRS (*laboratoires propres*), and another 250 shared with another organization, often a university (*laboratoires associés*).

The laboratories are distributed all around France, but by far the largest single geographical group is formed by Paris and its immediate locality — something which simply reflects the unusual dominance of the capital city of France over the rest of the country, in all sectors of life. However, CNRS has set up regional administrations, with a certain degree of power, and the new government is making an effort to identify new research "poles" outside Paris — or





How the 1982 CNRS budget is being divided up. Life sciences get the lion's share. "Big science" is not as prominent as might be expected as budgets are shared in these subjects with CEA and CNES.

bodies. This year INAG receives FF 115 million, IN₂P₃ FF 576 million from within the CNRS budget. The structure is said to make the management of "big science" easier; certainly, battles over funds for expensive equipment are fought at a higher level in the CNRS hierarchy than might otherwise be the case.

The total CNRS group budget of FF 6,000 million represents an increase of 25

per cent over last year, a general increase which hides even larger rises in some sectors. General programme support and small laboratory expenses has risen 36 per cent from around FF750 million to over FF 1,000 million. Salaries take the biggest single chunk: FF 3,900 million. Increases over 1981 are smallest for large equipment and computers (just over 10 per cent). Against these increases weigh the current

inflation figure of 14 per cent; the increase in the dollar against the franc (up 30 per cent over 1981); and the freeze on FF 150 million of CNRS funds (its share of the 25 per cent new money freeze — see first story).

New posts have also been created: 447 researchers and 193 engineers, technicians and administrators.

The new funds are not distributed equally among all research sectors supported by CNRS. The biggest fractional rise goes to an interdisciplinary programme of solar energy and primary materials (PIRSEM), which will get FF 10 million, over three times as much money as its predecessor PIRDES.

Besides that exceptional case, applied physics (physical sciences for the engineer) is most favoured: it receives a 40 per cent rise. Life science gets 38 per cent, earth science 36 per cent. Life science gets the biggest share of the new research posts (127) but then it also has the biggest share (2,500) of the total research staff of CNRS. The budget increases will be used for four principal purposes. First, to re-equip laboratories to "bring their means back to an internationally competitive standard". General laboratory spending suffered a severe decline between 1972 and 1980, say CNRS staff. The previous science minister, Pierre Aigrain, won a substantial increase in the general science budget in 1981 (8 per

Pressing problems for Payan

THE major issue facing the CNRS director-general, Jean-Jacques Payan, and president, Claude Fréjacques, is the reorganization of CNRS — to which the new government and CNRS directorate are committed.

At present, CNRS is structured around a remarkable scientific parliament called the *Comité National*, which consists of about 1,000 people, roughly two-thirds of them elected every four years from CNRS laboratories and groups and one-third nominated by the government from among scientists, industry and the administration. Researchers, engineers, technicians and administrators are all represented. The *Comité* is divided into 41 sections according to discipline. In spring these sections advise on individual careers and promotions, and in autumn they consider the prospects of groups and laboratories.

This advice passes to the eight scientific directors of CNRS, who are usually career scientists who spend a period of 4–8 years on part-time leave from their posts to direct a sector of CNRS activity; and then to the "double head" of CNRS, the director-general and president. Ultimately, major decisions are taken by the governing body of CNRS, the government-nominated *Conseil* of which Fréjacques is president.

To those interested in increasing "democracy" in French science — which

means effectively reducing the power and influence of the scientific directors of CNRS and, at the local level, of laboratory directors — the *Comité National* plays a central role. The scientific trade unions would like to increase their control and influence over



Payan (left) and Fréjacques face the unions

the parliament. Commensurately, it is felt that the powers of the parliament itself should be increased.

A struggle is taking place between these unions and the directorship of CNRS. The unions demand that nobody be elected to the *Comité National* unless he or she is accredited by a union — which represent mostly technicians, engineers and some younger researchers. Payan has sent a letter to laboratory directors to warn them of the threat and ask for their advice. One senior scientist cabled Payan to say he would resign all his CNRS

functions instantly if the unions were granted such power — and his reaction may not be untypical.

Professor Payan himself is shocked by the union militancy. He said recently that the thorough-going dialogue now under way in CNRS touched all levels, and that CNRS old hands had not experienced such a thing for a long time [since 1968, perhaps]. But, said Payan, whereas the union position against the previous right-wing government had understandably been "the most extreme possible", now was the time for a more comprising attitude. Was the socialist government not, after all, on the same side?

A second important — but less pressing — issue facing Payan concerns the relationship between CNRS and the universities. Under the previous government, CNRS was under the authority of the Ministry of the Universities, but now it is controlled by the Ministry of Research and Technology. Since CNRS already creams off some of the best scientists from education, this shift has led the universities to fear a deepening of the class divisions between university and CNRS laboratories.

This fear is further increased by plans to give CNRS a role in the education of researchers. But, says Payan, a break between the universities and CNRS would be a bad thing. CNRS will cooperate with the universities, he says — but, on the other hand, the universities have a long way to go . . .

cent in real terms, he claims), and this year that trend is accelerated to 25 per cent in real terms.

Second, the aim is to bring to fruition certain plans for large machines — the heavy ion nuclear accelerator (GANIL) and a French dedicated 800 MeV synchrotron radiation source, Super-ACO, at Orsay (in collaboration with the Commissariat à l'Energie Atomique, the Ministry of Universities, and the Université Paris-Sud). Increased support will be given for the reactor Orphée and the trilateral high flux neutron beam reactor at the Institut Laue Langevin in Grenoble.

Third, new themes of CNRS support are to be established, particularly through the special programmes called Actions Thématiques Programmées (ATP) under which CNRS laboratories and groups compete for extra money available for

work directed to particular objectives — which are becoming increasingly technological. This year ATP cash has crept up to nearly 10 per cent of the research money otherwise available to CNRS laboratories.

Finally, FF 8 million have been set aside for a programme of so-called "young groups", which is designed to give younger researchers a chance to create their own groups earlier than would otherwise be the case. The programme also gives CNRS the chance to go head-hunting in some of the smaller universities for new talent. The name "young groups" may be a bit misleading, however: the eight "young" group leaders recently selected by the life sciences sector of CNRS are all about 40 years old. "We hope we can bring the age down somewhat next year" said a CNRS spokesman. □

Medicine hot, agriculture cool

CONTRASTED with the scale of the CNRS, the institutions which support health, medical and agricultural research may seem small, but they have a significant impact on research. INSERM, the Institut National de la Santé et de la Recherche Médicale, supports 1,500 researchers (compared with 2,500 in the life sciences sector of CNRS alone). Even so, this is over a third of French biologists, and the means at INSERM's disposal are not negligible: over FF 1,000 million this year. Since the CNRS life science budget is just under FF 1,000 million for nearly twice as many researchers, INSERM scientists can count themselves rich.

INRA, the Institut National de la Recherche Agronomique, is also substantial — although until recently it had appeared to have been neglected by the new government. INRA has 200 researchers and a 1982 budget of FF 1,400 million. INRA is unusual in being highly decentralized, as befits an agricultural agency, with laboratories all over France and also in Corsica and Guadaloupe.

INRA politics, for the moment, are relatively quiet: INRA's director-general, Jacques Poly, has not yet felt obliged to resign over some difficulty or other with the new minister of science — unlike his former colleagues at CNRS and INSERM, who did just that. But perhaps that is because the ministry has hitherto paid little attention to agriculture. Earlier this month, however, the establishment was announced of a new department at the ministry of research and technology, to be concerned with agriculture and food.

Chevenement appears to have noticed that the agroindustry employs 3.5 million workers, and that its activities concern every consumer in the country.

The new department will assess the present state of research and development in this area in France; define priority

research areas; stimulate relevant research in small industries and businesses; extend the links between INRA and industry; establish the needs for training and recruitment; and take account of the needs of developing countries.

What Professor Poly makes of this has yet to be seen, but meanwhile the new director-general of INSERM, Professor



Lazar — back to the watering can?

Philippe Lazar, is settling in after his predecessor's resignation. That gentleman, Professor Philippe Laudat, was very oblique about his reasons for resigning, but the speculation is that he feared that his efforts to concentrate INSERM funds on the best groups, so avoiding what he described as "an even sprinkling" of money regardless of quality, would be reversed by the new government. As what Laudat identified as "best" often meant groups working in molecular biology, INSERM researchers in that area are worried.

For Professor Lazar, if anybody, would be the one to apply the politics of Chevenement to INSERM: he was rapporteur for the National Colloquium of Science and Technology in January. So what does he plan to do?

Laudat certainly had trouble with the scientific trade unions, with his determination, as he put it, to open up INSERM

research; and Lazar will clearly be more sympathetic to union views. Lazar sees no reason to be so selective with basic research money as was Laudat, because research funds for all basic science are guaranteed to rise 13 per cent in real terms (according to Chevenement's forthcoming law).

"We should try to mobilize almost all research units" says Lazar, "and be a little more confident in the ability of all groups". It is possible to be too elitist, he says. How is one to detect which research unit is going to be successful, as opposed to those already at the top?

Lazar intends to apply a policy of *a posteriori*, rather than *a priori* assessment: each of the INSERM units will be expected to present a programme every two or three years in which it expresses its needs; INSERM will then respond. This will save laboratory directors time in their constant efforts to raise money. Each unit will be assessed at this one time by INSERM's eight specialized committees and scientific council, which will give advice on the closing of certain units and the opening of others. But the specialized committees should "follow each laboratory more closely and interfere more".

It should be easier than at present to change the directors of laboratories, says Lazar. As things stand, a director is appointed for six years — after which he or she is assessed and, almost without fail, re-appointed. "The general rule should be that a director should change", says Lazar.

Lazar also feels that the links between the INSERM administration and its scientists are too weak, and he would like to make use of the 225 committee members more than the present twice a year; and he wants to try to improve INSERM's links with the outside world, including CNRS, the Institut Pasteur, the universities, and institutions beyond France.

CNRS and INSERM

Lazar would like particularly to rationalize the overlapping relationship with CNRS, a desire shared by CNRS director of life sciences, Professor Roger Monnier. Sometimes both INSERM and CNRS support the same laboratory in different ways, and neither knows what the other pays. Lazar favours joint committees set up between CNRS and INSERM.

Of molecular biologists relying on INSERM grants, Lazar says "they may be afraid but I am not". Molecular biology will receive "at least 13 per cent" in real budget rises in coming years — as a basic science — and in so far as the work is linked to biotechnology, nearer 30 per cent. For Lazar will countenance certain *programme mobilisateurs* which will receive more than the average attention. Biotechnology will be one; also the social sciences of health care. Clinical research, generally thought to be weak in France, will also receive special attention, by "opening it up to other research". Lazar intends to hold a meeting to illuminate that issue. □

Biotechnology — gearing up

BIOTECHNOLOGY — including the most basic molecular biology — is to be one of the principal axes of M. Chevenement's French technological revolution. Moreover it is the first of his ministry's programmes to get off the ground, because its main points are identical to those followed by the previous government. This is not least because the biotechnology adviser to the previous government now spearheads Chevenement's biotechnology department at the ministry. He is Professor Pierre Douzou, a well-known microbiologist at the University of Paris. The only obvious change from the earlier policy seems to be the fivefold budget increase.

The main weaknesses in French biotechnology have been identified as a woeful lack of microbiologists, a poor system for training genetic engineers, too little work on plant cell biology and weak links between the worlds of research and industry. The genetics of nitrogen fixation is also thought to be poorly represented in France; and lastly, the French pharmaceutical industry is particularly weak in the antibiotic market, and so has little experience in the sophisticated fermentation technologies that antibiotic production requires. On the other hand,

France can boast some world-class groups in molecular biology, particularly at the Institut Pasteur and the University of Strasbourg. The lack of microbiologists is perhaps surprising in the country of Louis Pasteur, but just as in other countries, French biologists have followed the trend towards concentrating on "laboratory" species such as *Escherichia coli*. To correct the trend, CNRS is planning to set up new groups or laboratories working with species such as *Pseudomonas* or *Actinomyces*, and, they say, they are ready to import the right group leaders from abroad. French universities are also preparing to establish professorships in microbiology. Also, plans are almost complete at CNRS to establish a new laboratory of plant genetics in Strasbourg, at a cost of around FF40–50 million, which will employ 100 scientists.

At the Institut Pasteur in Paris construction is going ahead on a new building devoted to biotechnology, to open in 1984–85, according to a decision taken by the previous government. Among other things the building will provide facilities for 5–10 litre fermentation trials and will have a laboratory for the development of hybridomas.



Science and the scientific are chic right now in France: witness this advertisement for DNA cream from a French magazine.

At present the strong poles of CNRS biology with an impact on biotechnology are at Strasbourg (basic molecular biology and plant science), the Institut Pasteur (all fields), Toulouse (selected by Aigrain to be a special centre for biotechnology), Marseilles (laboratories of immunology

The Pasteur leads the way

THE Institut Pasteur, founded by Louis Pasteur in 1888, three years after his development of an antirabies vaccine, became and remains one of the most important biological laboratories in France; and now, it is also taking a leading role in the development of French biotechnology.

This is happening both at the political and practical level. François Gros, the previous director, has taken a deep interest in biotechnology, and in his last three years as director (he resigned at the end of last year) had begun a slight shift of the Pasteur away from human and animal pathogens (its traditional interest) towards microorganisms of potential economic importance. Now he has become science adviser to the Prime Minister, Pierre Mauroy, where he has considerable influence. Moreover, the National Colloquium on Science and Technology, the great conference of scientists and public that has been one of Chevenement's most imaginative achievements, was Gros's idea. Gros organized and chaired the colloquium, so endearing himself to the new minister of science as well as the Prime Minister.

On the practical side, the Pasteur is now seen in government circles as an excellent model of the combination of fundamental research with industrial and medical development, a kind of mix of priorities which can be traced directly back to the inspiration of Pasteur. The

Pasteur has a hospital. It has research which reaches the most fundamental levels of biology (three of the six French Nobel Prizes since the war went to the Pasteur). And it has three distinct ways in which it tries to exploit its research.

There is Institut Pasteur Production, which has a first call on potentially profitable discoveries at the Pasteur; Groupement Genie Genetique (G3), which seeks directly to develop profitable ideas, which it would then license to industry; and a highly differentiated and active system for linking Pasteur staff with industry, making a profit for the Pasteur and aiding the biotechnology industry at the same time.

The original Institut Pasteur building of 1888. Pasteur's tomb (see cover) is on the site.



and bacterial chemistry), Compiègne (enzymology and enzyme fixation) and to a lesser extent, Lyons and Montpellier.

Douzou outlines the principal objectives of the ministry as first, to improve training and education in the field; second, to transfer information more efficiently from the laboratories to industry; and third to establish "a multitude of sites" in France as small technology centres, which would embody perhaps four or five researchers, several engineers, and technicians, each working on some pilot scheme. These technology centres should be encouraged in industry as well as in the public sector such as CNRS and INSERM, says Douzou.

In that public sector, Chevènement has said that such laboratories would be judged by the patents achieved, by licences sold to industry and by their general impact on the industrial sector rather than by their scientific productivity *per se*.

However, development work in biotechnology can be very expensive, and France is certainly willing to consider international agreements and programmes at that level. They should be bi- or tri-lateral and specific, however, rather than conducted through an intermediary like the European Commission in Brussels. □

Industry cloning

FRENCH industry boasts a handful of main actors in genetic engineering. They are Transgène, a venture company with a small amount of government support based in Strasbourg; Genetica, a genetic engineering wing of the chemicals giant, Rhone-Poulenc; G3, a group based at the Institut Pasteur in Paris and supported by government money through CNRS, INSERM and INRA and (51 per cent) by the Pasteur itself; and the nationalized oil company Elf Aquitaine. Elf is to set up a FF100 million biotechnology laboratory in Toulouse, and through its pharmaceutical subsidiary Sanofi it has a 51 per cent share in Institut Pasteur Production, the company with first claim to the development rights of Pasteur Foundation discoveries.

None of these companies except Institut Pasteur Production (which is an older company with a strong interest in vaccine production) yet has a marketed product, and all are keeping their plans fairly close to their chests. But Transgène, at least, is known to have commissioned reports on the microbial extraction of minerals from sea water and to be considering a special effort on plant cells — stimulated by CNRS plans to make Strasbourg a focus of plant genetics. Transgène's director, Jean-Pierre le Coq, is also looking closely at what the new biology can do in traditional biological industries such as food processing — where, he says, the field is huge and the competition less.

Telecommunications — late start

FIVE years ago, the French ministry of telecommunications, PTT, launched an ambitious plan to improve the services offered by the public telephone network. Since then, massive investment has increased the number of telephone lines from 7 million to 16 million. When he presented his budget to the assembly earlier this year, Louis Mexandeau, the new PTT minister, indicated that he wishes to continue the modernization albeit at a slightly slower rate. He is looking for a further FF 25,000 million a year for the next five years to give a telephone to everyone who wants one by 1985.

As PTT admits, the success of the modernization so far is partly due to the initial small size and poor state of the telephone network. France has been able to opt for the latest technology and avoid the problems inherent in modernizing an out-of-date, yet extensive, system. Hence, for example, France has been able to move over to fully electronic exchanges more rapidly than some of its European neighbours. The plan now is to digitize the network fully by 1990.

To achieve its goals, however, PTT has had to lean heavily on industry. Although the result has been to increase the international competitiveness of the French telecommunications industry, there have been problems along the way. Pierre Aigrain, former science minister who has now returned to his old haunt Thomson CSF as research director, mentions the difficulties caused three years ago when PTT changed suddenly from installing partially to fully electronic exchanges.

The switch meant that Thomson had to reduce its workforce, a task it has still not managed to complete. Thomson was also at a disadvantage because, unlike its competitor, CIT-Alcatel, it did not receive state help to develop electronic exchanges. PTT supported development in its own research centre, the Centre National d'Etudes des Telecommunications (CNET), and then licensed the product for manufacture by CIT. The CIT product works well, but Thomson is still having software problems.

(The PTT supports most of its research on electronics at the CNET laboratories in Grenoble, which, like the laboratories of CNRS, have been asked to step up the number of researchers employed by 4.5 per cent a year. The ministry of research and the PTT now want the Grenoble laboratories to develop into a major centre that will drive not only telecommunications but also the broader electronics industry.)

Switching to optics

The French have also been fortunate in their choice of technology. By choosing a time division switching electronic exchange, they have suffered fewer technical problems than say Britain with its choice of System X. But installing

electronic exchanges is just the first step in PTT's vision of transforming the network into a modern system for digital rather than analogue transmission.

Like its European neighbours, it is nibbling vigorously at the bait of optical fibres. Thus a 12 km optical link has been installed between two telephone exchanges in Paris and two more links are planned.

Optical fibres are still too expensive for widespread introduction, largely because the signal has to be boosted at regular intervals of about 30 kilometres. The PTT will be waiting until the price comes down to about FF1 per kilometre. But Saint-Gobain and Thomson CSF seem confident that the market will expand soon. Recently, they formed a joint company, Fibres Optiques Industries, with Corning Glass, to manufacture 10,000 km of optical fibres a year, half planned for export.

Optical fibres are also being connected to 2,000 households in Biarritz in the south of France for an experiment, beginning in 1983, that will give each household access to new telecommunications services such as videophone, videotex and cable television, via a telephone linked to an ordinary television screen. A further experiment to bring cable television via optical fibres to the people of Lille is also planned. The aim is to test the usefulness of new technologies and the information they bring to individuals and businesses before launching into full-scale development.

Planners seem particularly keen to bring new telecommunications technology to the business community. In 1983, French companies will be able to make use of the services of a satellite more or less devoted to business communications when Telecom 1, a satellite being built with PTT backing under the direction of the French space agency, CNES, is launched. In addition to providing business links, Telecom 1 will free France from the expense of leasing channels on Intelsat satellites for telecommunications links with its overseas territories.

The Mitterrand government clearly shares the view of its predecessors that modernization of the network is essential. But where the old and the new policies may part company is in a strategy for widespread introduction of *télématique* — the uniquely French word for the information systems created by linking computers via telephone lines — into homes. The new government is sceptical of the economics of the Giscardian plan to give every home a *télématique* terminal. And Jacques Dondoux, the new director of telecommunications, has made a concession to public anxieties about the social impact of *télématique* by agreeing to set up a users' group to discuss the issues.

The previous plan to install and experimental electronic telephone directory into every home in Ille et Vilaine in Brittany has

thus been modified to give householders the right to refusal. Householders in Velizy, a small town near Paris, are also to be allowed to choose whether to participate in an experiment to monitor the use of Teletel, the French public viewdata system. The results of both these experiments, which began last year, will be fed into a national debate on the new technology.

PTT plans to finance its future investment programme in much the same way as it has in the past — from earnings and money borrowed abroad. As the network grew, the proportion of investment raised from receipts grew from 43 per cent in 1977 to 74 per cent in 1980. The hope is that three quarters will come from cash flow.

Thus the pressure on the public purse should continue to be minimal. But the Mitterrand government, in its eagerness to offer the benefits of new telecommunications technology to everyone that wants it, has already suggested that PTT offer special tariffs to certain groups, for example the elderly. If it sticks to its guns, then PTT will be looking for public money to make up the deficit. **Judy Redfearn**

Microcomputers for all

WHILE other countries agonize over the social impact of new information technologies, France is preparing to take the bull by the horns in the form of a new centre set up last October with an annual budget of FF 100 million. The Centre Mondial, as it is known, is the brainchild of Jean-Jacques Servan Schriber, author of the book *The World Challenge*, who believes that microcomputers can wipe out social inequality by bringing information to all. Last week, the Centre Mondial held its first assembly.

Professor Nicholas Negroponte, professor of computer graphics at Massachusetts Institute of Technology is managing director. He will be supported by a board consisting of 12 French cabinet ministers and a group of French and foreign scientists. Around 50 researchers will be appointed this year and a further 50 by the end of 1983.

The idea, however, is not simply to get computer scientists to design micro-

computers. The public must also be involved in testing new and existing information technologies. Hence the centre will be establishing offices where passers-by can test out various wares. The ultimate aim is to feed public reaction into the design of a powerful microcomputer, costing no more than £100, for personal use.

In the meantime, the centre will study social questions such as the human-computer interface and the value of information technologies to members of different cultures. Two projects have begun. One, to bring microcomputers to a quarter of Marseilles in collaboration with the University of Aix at Luminy, will test the French response to the new technologies. But the other, to train young people from Senegal in the use of microcomputers, will be used to assess the usefulness of the new technologies to those living in developing countries.

Judy Redfearn

National trend in space

THE enthusiasm of the Mitterrand government for science and technology has not been lost on the Centre National d'Etudes Spatiales (CNES), the French national space agency. CNES has been promised 18.7 per cent more money, in real terms, in 1982 than in 1981. The lion's share of the FF 3,000 million budget for this year, which makes CNES the largest national space agency in Europe, comes from the research ministry. But other government ministries which must contribute towards development of services they wish to use have also stepped up their payments. These include the ministry of defence, which wishes to launch satellites on Ariane, and PTT (the telecommunications ministry) which is contributing towards the cost of Telecom 1, a national telecommunications satellite.

For the time being at least, the new government seems keen to support the space policy of the old. Thus, last October it formally approved CNES's involvement in four new projects of the European Space Agency (ESA) and a second national remote sensing satellite, Spot 2. CNES will have no difficulty spending this year's extra money according to Professor Hubert Curien, the centre's president, largely because Spot 1, the first national remote sensing satellite, is now entering the most costly phase of its development. But if the budget continues to increase over the next five years, as promised, how will it be spent? CNES hopes to persuade its own government and those of its partners in ESA to embark on projects that rely less than in the past on the good will of the United States and the Soviet Union for their success.

Since CNES was created 20 years ago this month, it has followed a policy of multi-lateral and bilateral cooperation that has benefited both national space industries and the space science community. Hence, membership of ESA has given French engineers the opportunity to participate in the development of their brainchild, Ariane, a project that would have been too costly for national development. And the policy of bilateral cooperation with the Soviet Union as well as the United States and individual European countries has given the French space science community perhaps more opportunities than its counterparts in, say, Britain and Germany.

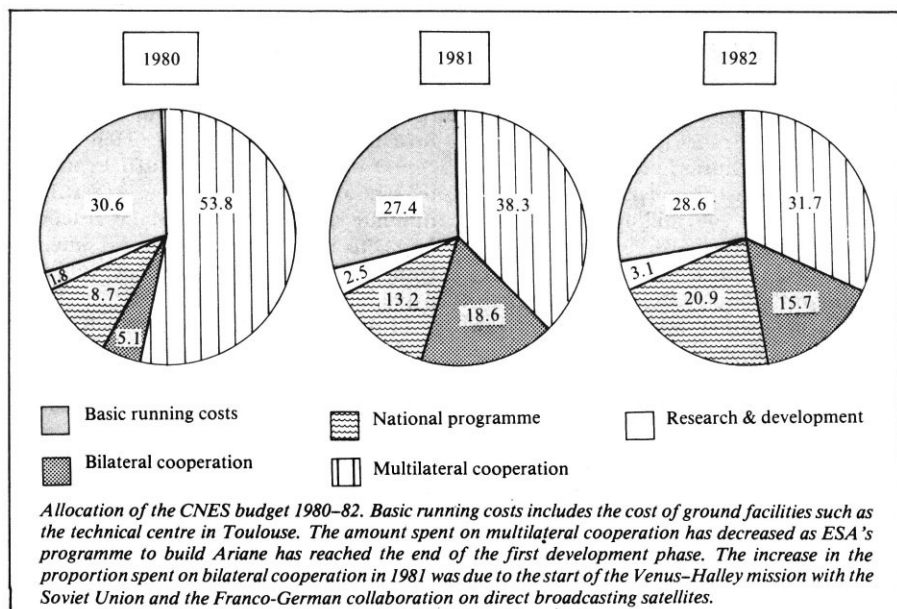
International collaboration, however, has not always run smoothly. CNES still remembers, for example, NASA's decision to withdraw from participation with ESA in the International Solar Polar Mission. Joint projects with the Soviet Union have caused similar frustrations. And the decision of the Soviet space agency to extend its next mission to Venus to include Halley's comet meant that CNES had to abandon plans to send a probe into the atmosphere of Venus.

CNES now wants to shake off the shackles of being the junior partner in collaborations with the two space powers, whilst retaining a strong commitment to Europe on the grounds of necessity. It is following two paths towards its goal. The first involves persuading the French government to support an enlarged national space programme, especially in science. And the second involves persuading the members of ESA to develop an ambitious launcher as a successor to the current series of Arianes.



Hubert Curien — keen to promote ESA as senior partner in bilateral projects

The national space programme centres on the development of applications satellites, five of which will be launched in the next three or four years. These include a telecommunications satellite, Telecom 1, due for launch in July next year; a direct broadcasting satellite, TDF 1, identical to West Germany's "TV-sat"; and two Spot remote sensing satellites for launch in 1984 and 1985. But when these projects come to an end, CNES may well decide that most future research and development in applications, especially telecommunications, should be hived off to national industry which has already developed more expertise in space technology than many of its European counterparts.



The field could then be open for the first national scientific satellites since 1975. The French space science community, enthused by the possibilities, thrashed out about ten proposals at a meeting last year for wider discussion at the national colloquium on science and technology held in Paris last January.

CNES is studying the feasibility of all the proposals, some of which will eventually be put to ESA. But two, a gamma-ray observatory, Sigma, and a wave altimetry experiment, Poseidon, have been singled out for national development. The reasons for choosing them were their scientific merit and the urgency with which they would need to be developed. The plan is to launch Sigma at the end of 1985 on the first qualification flight of Ariane IV, the latest

version of Ariane to be approved, and to carry the Poseidon experiment on board Spot 2.

CNES now has the task of persuading the French government to find the money for these projects, which could entail almost doubling the space science budget. Financial pressure could be eased, however, if other countries could be persuaded to participate as junior partners. Discussion over collaboration on Sigma has already begun with scientific groups in Italy and Britain.

Thus, with national scientific projects, CNES hopes to regain the power of decision it believes it has lost in many of its international undertakings. Professor Curien would like ESA to follow suit. France had supported moves by Germany at the end of last year to increase ESA's science budget by 20 per cent after 1983. Other members states vetoed the proposition but CNES now hopes that if some of its scientific proposals cannot be afforded within ESA's mandatory science programme, then sufficient member states can be persuaded to participate on a voluntary basis.

Professor Curien believes that ESA too could gain greater control over joint projects with non-European countries if it initiated attractive projects in which, say, US scientists could be invited to participate. His proposal, however, has met with resistance from some members of ESA who fear that US participation could swamp what should still be essentially European projects. The outcome may depend on the findings of a committee of the European Science Foundation.

The strongest evidence of the importance that CNES now attaches to national and European independence in space, however, is to be found in its belief that Europe must build a new generation of launchers that will rival the descendants of the US space shuttle in the 1990s and beyond. At a meeting organized by CNES earlier this year, Yves Sillard, then

director-general, urged representatives of the European space industries to come up with proposals within the next two years. The French idea is to develop an advanced launcher consisting of the first stage of Ariane IV with a powerful cryogenic motor for the second stage. A third stage could be added later. CNES engineers plan to put a proposal along these lines to ESA by early 1984 with the aim of reaching a decision in sufficient time for an operational launcher by 1994.

The French philosophy is to design a flexible system that could launch the future generation of applications and scientific satellites and give Europe independence in new space applications such as material processing. The dilemma is knowing whether Europe will need men in space or whether it should design fully automated systems. CNES engineers are trying to avoid the problem by designing a partly recoverable, automatic system, initially compatible with Ariane IV, that could be converted to manned spaceflight, using the more advanced launcher, when and if the need arises.

Two separate but related projects are under study. The first, Star, a geostationary satellite that would maintain permanent contact between satellites and a ground station by relaying signals between them, may be affordable nationally. The second, Solaris — consisting of one or two Star satellites, an automatic space station in low Earth orbit and an automatic recoverable servicing vehicle — would be costly enough to justify collaboration within ESA. The recoverable vehicle would be of sufficient power to ferry, for example, materials for space processing between earth and the space station. It would also be capable of carrying out routine maintenance and repairs on the space station. Fitted with a suitable boost motor, it could be sent on servicing missions to satellites in geostationary orbit.

The development of both Star and Solaris demand research on space robotics and advanced control systems which CNES and ESA are currently supporting, albeit on a small scale. Ideas for putting men in space centre on developing a suitable recoverable servicing vehicle and space station for the Solaris system and a more powerful launcher. CNES is supporting feasibility studies of a small manned vehicle, Hermes, and a cryogenic motor with up to 100 tonnes of thrust for the second stage of the advanced launcher.

The French government, with its plans for dragging French science and technology to near the top of the international league and with its penchant for national independence through international collaboration, seems set to go ahead with these plans. But the success of CNES's proposals will depend critically on whether other governments, which may prefer to leave the expense of advanced launch systems to NASA, can be persuaded to play ball.

Judy Redfearn

Da ili nyet

THE programme to put a French astronaut in space aboard Soyuz-Salyut later this year contributed to CNES's mistrust of bilateral deals when the Soviet space agency refused to release details of the spacecraft for reasons of military secrecy. French engineers then had no option but to guess the specifications for the instruments they are putting on board. In the event, the Soviet space agency allowed French technicians to travel to Moscow for consultation provided that they did not enter the buildings where the spacecraft were being built. But in general, when French engineers ask whether their guesses are correct, the Soviets simply reply "yes" or "no". The result, according to Professor Curien, is "trial and error with rapid convergence". Added to these technical problems is the growing row among French space scientists over the political wisdom of Soviet collaboration. Many are now objecting because of events in Poland and the human rights issue.

Judy Redfearn

Taking a nuclear power lead

"ONE reactor, one producer, one seller": that is the structure of the French nuclear industry, and according to one observer it is a prime example of how France has managed to mobilize its technical resources towards particular goals. Whether this technocratic centralism will survive a socialist government has yet to be seen, but the nationalization of large sectors of industry, and the grand "mobilization programmes" of the minister for research and technology smack not so much of a change as a clarification of previous government positions.

The reactor is the pressurized water reactor (PWR), built under licence from Westinghouse in the United States; the producer and seller the 5,000-strong company Framatome, now geared up in its pressure vessel and steam generators shops at Le Creusot and Chalon to produce six 1,300-MW reactors a year. Mitterrand did not nationalize Framatome. It was already in effective government control through its sole French customer, Electricité de France (EDF) and its principal collaborator in research, the Commissariat à l'Energie Atomique (CEA).

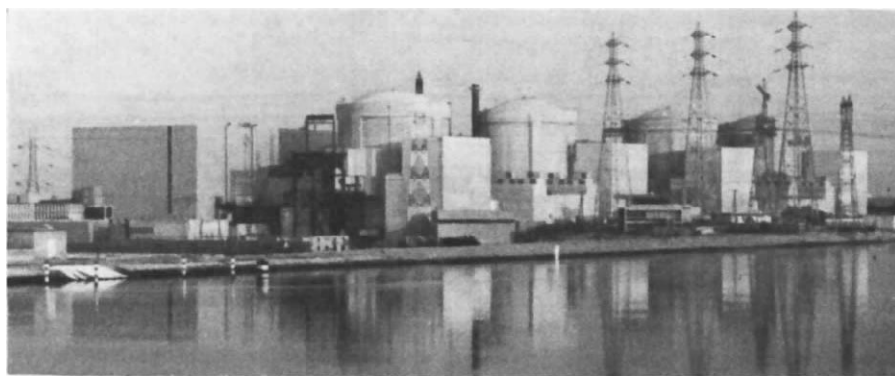
The main impact on the Framatome programme has been the reduction in the EDF order from a possible nine reactors over the next two years to six, which leaves Framatome with a large surplus capacity and a hunger for export orders. Framatome has sold one reactor a year for export over the past eight years: two to Belgium, both to come into operation this year, two to South Africa (to operate next year), two to Iran (although the orders were cancelled by Khomeini with the plants half-completed) and two to Korea. The sale to Korea was a considerable achievement because Korea's previous reactors had been constructed by US companies.

Nucléaire, s'il vous plaît!

A SLIGHT majority of the French people is in favour of nuclear power, a poll conducted just before the October 1981 energy debate indicated. Some 45 per cent are in favour, compared to 40 per cent against and 15 per cent who don't know, in a sample of over 2,000 people. On the extremes, 16 per cent were "definitely" favourable, and 17 per cent "definitely" unfavourable.

One surprising result which might give the government pause, however, was that 64 per cent were in favour of a referendum on nuclear power — an election promise that was subsequently dropped. Another surprise: nuclear power is more supported on the political left than on the right. Compared with the average 45 per cent in favour, 51 per cent of those who voted communist and 52 per cent of those who voted socialist favour nuclear development.

Framatome has also put in tenders for two reactors for Taiwan and two for Mexico — and if it were not for the world recession would be confident of orders. The dollar rose by nearly 30 per cent against the franc during 1981 (from FF 4.5 to FF 5.8), giving Framatome a distinct price advantage — which is enhanced by the company's ability to spread its overheads over a large national programme. However, Mexico, at least, concerned about its economic situation, is beginning to have doubts about whether it needs the 20 reactors it was planning to build by the year 2000, and the same fear appears to be gripping other developing



Pride and glory reflected — four of France's "production line" PWRs, at Tricastin

states interested in nuclear power. So Framatome may be forced to look to the home market.

In France, Framatome is now building 26 reactors with a total power of 28.6 GW electric, 16 at a nominal 1,300 MW and 10 at 900 MW. Another thirty units, accounting for 21.8 GW, are already in operation. The smaller reactors are effectively the original Westinghouse design, as modified by Framatome; but the 1,300-MW plants are almost completely French, and a new 1,400-MW design, called "N4" and now on offer to EDF, is totally so.

Going it alone

The Westinghouse licence expires this year and then Framatome will be officially on its own — apart from certain research agreements which will continue — but already the sluggishness of the US nuclear programme has given Framatome a commanding lead. According to Framatome's technical director, M. Michel Coudray, the company began work on the 1,300-MW design in 1975. By then Westinghouse had already sold two 1,300-MW systems to a Texas utility, and Framatome believed that it would be able to benefit from Westinghouse experience before putting the final touches to its own 1,300-MW plants. But in the event, construction of the Texas reactors has ground almost to a halt, due to quality control problems on site and concern on the part of the Nuclear Regulatory Commission. The result, said

Coudray, is that while the Texas reactors are not likely to go critical before 1984 (three to four years late), the first Framatome 1,300-MW reactor — at Paluel — should be connected to the grid early next year. Framatome will thus have taken less than six years to build it, whereas Westinghouse will have taken more than ten years for a similar reactor.

In part, this achievement can be put down to standardization. Framatome has not had to make substantial changes in design in mid-stream to meet changing safety regulations. Apart from the first five reactors, which were fairly variable — Framatome was on its learning curve — there have essentially been only three designs: a group of 16 reactors of 900 MW which began construction in 1974–76; a

second group of 12 begun in 1977–81; and the 1,300-MW reactors, begun in 1977 in parallel with the second tranche of 900-MW systems. The result has been that Framatome has been able to place long orders for components, which gives a guarantee of supply, and streamlines production processes. (One danger, however, is that mistakes, once made, are propagated over many systems; see p.301.)

With this powerful production system now established, Framatome would, of course, like a long list of orders; but even under the unbounded nuclear enthusiasm of the previous government, orders came only in occasional handfuls. Furthermore, EDF is now producing 40 per cent of its electricity from nuclear power, and while it aims for 60 per cent nuclear by 1985 (compare Britain's present meagre 11 per cent contribution), there is finally a limit to the proportion of an electric power network that can be driven by nuclear systems. The reason is that national power demand fluctuates by as much as 40 per cent during a working day, and nuclear power stations are not easy to turn on and off: they are said to provide "base-load power". So already, EDF has insisted that Framatome consider the problems set by imposing a ten per cent peak to peak fluctuation on demand.

This so-called "daily load follow and frequency control mode" leads to a number of new technical problems, caused by the high frequency at which the control rods have to be driven in and out (up to

much faster with Framatome, and now France can gloat over the difficulties facing the British nuclear industry — particularly over the design and cost of safety systems. “We are very sorry for the British” said Coudray. “You can increase the cost of a plant by a factor of two if you are not careful with what you do about safety”. According to M. Rémy Carle, a director at EDF, the cost of a Framatome 1,300 MW PWR station is now about FF 5,000 per kilowatt (that is, about £600 million in total) — a price that does not include decommissioning, and assumes a reactor on the coast, but includes all other costs. By contrast, at the recent announcement of the completion of the work of the British PWR task force, whose objective was essentially to reduce the cost of safety systems on the PWR, the figures being quoted were more like £1,000 million.

According to Coudray, the problem is that the British have adopted a four-loop, independently pumped emergency core cooling system (for use in the event of the most serious possible emergency, a “loss of coolant accident” or LOCA). Such a system is easier to explain, and “nicer



Coudray of Framatome — unlike the British, not troubled by a suspicious public

intellectually” that the French safety system, says Coudray. The British system takes five minutes to explain whereas the French takes 3-4 hours — but the French system will do everything the British one will do a much lower cost. Britain has probably adopted the “transparent” system for political reasons, thinks Coudray, to satisfy a suspicious public.

How then has France got so far ahead with its nuclear programme? The research programme undertaken by Framatome in the early 1970s, and which was in full swing by 1975, was crucial to success. A licence brought information; but understanding requires research, said Coudray. Research enabled Framatome to move away from and improve on the Westinghouse design. The exact scale of the French research programme has not been revealed, but it matches anything that Westinghouse has done in the United States.

The company does its research under four framework agreements which allow very rapid changes of direction and commitments of money without the need for constant negotiation and renegotiation. Framatome has its own unique programme

All they're cracked up to be?

YOU couldn't really have a better recommendation. “Now's the time to buy from the French” said Dr Walter Marshall, chairman of the United Kingdom Atomic Energy Authority, recently, when describing the French design for a pressurized water reactor (PWR). Dr Marshall heads the PWR task force which recently tidied up the design for a British PWR, built under licence from the American company Westinghouse. However, the recommendation is somewhat backhanded.

“The French have been caught with their pants down”, said Marshall, referring to a series of cracks discovered in the reactor pressure vessel and steam generators of a number of plants three or four years ago. The result is that the manufacturers, Framatome, are now taking every possible precaution to avoid such potentially dangerous flaws, even though their own “pessimistic” calculations had shown the existing cracks to be unimportant.

Marshall says he has every confidence in Framatome's calculations and experiments, and believes that it has done a “very thorough job”. French safety authorities will impose a requirement to inspect existing cracks every time a reactor is shut down for servicing (perhaps every 2-3 years), and Marshall — who made a deep study of the problems of cracking in reactor pressure vessels — believes this will be quite sufficient to prevent accidents.

This is despite the fact that some of the cracks, which appear in the black steel under the stainless steel cladding of the primary containment system, are difficult to observe. They are the ones in the shoulders of the inlet nozzles of the pressure vessel, where cool primary circuit water enters to extract heat from the core: it is simply physically difficult to reach them with the usual ultrasonic and eddy current detectors, unless the

whole of the core is removed first. But Marshall believes it would be adequate to monitor frequently only the cracks in the hot outlet nozzles, which are accessible, as a kind of statistical sample of crack growth, while inspecting the inlet nozzles less frequently — say every ten years — a policy which Framatome and EDF will probably follow.

Around 25 of Framatome's earliest reactors are affected by the underclad cracking problem — an example of how design continuity can lead to problems if the design or manufacturing process has a hidden fault. But the cracks may prove to be a very minor hitch compared with another problem which became apparent only this January — faults in the “broches”.

These are spring clips which grip and support the fuel cans at the top of the core, and some three years ago in Japan it was discovered that they can be subject to embrittlement caused, perhaps, by neutron bombardment. Until January there had been no evidence of this in Framatome reactors, but then a loose part of a broche was found circulating in the primary coolant in one of the Gravelines reactors. Now the loading of the latest Framatome reactor, at Chinon, has been delayed — according to some reports to test and inspect or even to change all the broches before the reactor is started.

Framatome staff do not understand yet what caused the Gravelines broche to break, and whether it was a unique or general fault. But if it was the latter, 37 reactors would be affected, so a crash programme on broche design and testing is now under way. The problem is compounded by the fact that the broches on working reactors will be highly irradiated and so very awkward to replace; but if faulty, they will have to be replaced, for broken broches could jam and interfere with the movement of control rods.

at Creusot-Loire, mainly on materials; a “very privileged and active” programme with the CEA (which is, incidentally, a 30 per cent shareholder in Framatome); a quadripartite agreement, under which one-third of the work is done in the United States and two-thirds in France; and a tripartite agreement among the French partners. Corrosion problems in the primary circuit and steam generators, among the main causes of the low availability of the Westinghouse reactors, were studied under the quadripartite agreement; but surprisingly Westinghouse and Framatome then diverged over steam generator design (see page 300).

The principal reason Framatome has been so successful, though, lies in the extra-

ordinarily tight organization of the nuclear industry in France — a legacy of the work of the previous industry minister, André Giraud. After the battle between CEA and EDF over whether the French gas-cooled or American pressurized water reactor should be chosen — a battle in which de Gaulle championed the CEA, which he had himself set up in 1945, and its gas-cooled system, and which was only won by EDF after de Gaulle resigned — Giraud picked up the pieces of the industry and dried the CEA's tears, giving it total control of the fuel cycle and the development of the fast breeder. He thus created the stable, three-legged organization — CEA, Framatome, EDF — which has put French nuclear power in a world-commanding position. □

Superphénix — great white hope

THE world's first commercial-scale fast-breeder reactor, Superphénix, should be generating 1,300 MW of electrical power for the French national grid by 1984. But is it, or will its successors be, economic? And will EDF order Superphénix II, which is already being designed?

Superphénix is in fact owned by an international consortium, NERSA, which is 51 per cent French with the rest largely divided between Germany and Italy (which has the larger share). NERSA is the customer of Novatome, a kind of fast-breeder companion to Framatome (which constructs the PWR). Framatome in fact now controls Novatome, which is also held 34 per cent by the CEA.

Superphénix is expensive: estimates vary, but the price is usually put at around twice that of the equivalent PWR. Of course, it is a prototype; it is alone on its site (PWRs are usually built in fours); and PWR construction is well-developed (Framatome is on the fortieth of its series). But there are some fundamental reasons why Superphénix should be more expensive: it has an extra cooling circuit — the second sodium circuit; safety systems are more complex, having to deal with the possibility of sodium fires as well as almost instantaneous nuclear shutdown in the case of coolant loss; and the reactor vessel is stainless rather than ordinary black steel.



Superphénix nears completion

In the long run, the fuel cycle costs should be lower because the fast breeder can generate its own plutonium by neutron bombardment of waste uranium from enrichment plants and depleted gas-graphite fuel.

This would also give France a degree of security in uranium supply. French-controlled uranium production is expected to peak at around 4,500 tonnes a year by the mid-eighties, but the PWR programme is expected to need around 8,500 tonnes a year by 1990 and 12,300 tonnes a year by the year 2025 (according to CEA figures). So France is in danger of becoming almost as dependent on foreign uranium supplies

as it is at present on foreign supplies of oil. The introduction of fast breeders, the CEA argues, could reduce this demand to as low as 8,000 tonnes a year in 2025 — provided a fast breeder programme were started soon.

There are two arguments for the fast breeder, says the CEA: that at a certain uranium price the fast breeder will become cheaper per kilowatt hour than a PWR, because of the former's breeding capacity; and that it represents an insurance against loss of uranium supplies. "And you expect to have to pay an insurance premium if you want insurance," says Michel Rapin, CEA director for nuclear applications.

The question of what balance should be struck between these two arguments is ultimately a matter for government and the electricity supplier, EDF, but neither shows any desire for haste although, says the CEA, France needs "a significant programme" of fast breeders by the end of the 1980s to make any impact on French uranium needs by the second decade of the next century.

Moreover, the reprocessing and other pieces of fuel-cycle plant necessary for a fast breeder programme are uneconomic unless built very large. So the CEA is looking for two options: first, a French programme of seven or eight fast breeders, the first of which should be ordered in 1986–87 or, perhaps more practical, an international collaboration on fast breeder construction on about the same scale.

Of course it would appeal to France if the international reactor were close to Superphénix II, and the CEA has already

been looking for partners. In Britain, UKAEA officials also believe in international collaboration on fast breeder programmes, and are contemplating agreements with either France or the United States. However, France was reported two years ago to be asking a price of £20–25 million for access to Superphénix technology, a price which Britain at the time rejected.

The reprocessing technology, whose efficiency in extracting plutonium is critical to fast breeder operation, is not seen to be much of a problem at the CEA. Already 15 tonnes of fast breeder fuel have been reprocessed through small fast breeder plants at La Hague (now closed) and Marcoule, and 380 of Superphénix's 400 or so fuel assemblies have been made of plutonium extracted through reprocessing. "We have closed the fuel cycle" says Rapin.

Moreover the breeding efficiency was "sufficiently positive". In recent years CEA scientists — 1,000 of whom work on reprocessing technology — have reduced plutonium losses during reprocessing by a factor of five. "But that is not exactly the problem" says Rapin. To get a lot of plutonium bred by a fast breeder, a thick depleted uranium blanket is necessary. But the thicker the blanket, the less the concentration of plutonium produced in the outer layers of the blanket. And a low concentration of plutonium means a high reprocessing cost. So the best balance has to be struck between the reactor breeding ratio and these costs. Costs, yet again, come back to the centre of the fast breeder stage, and there are certainly some in France who look at Superphénix and think of another costly marvel — Concorde. □

Nuclear power — how committed?

WHEN François Mitterrand was elected president of France on 10 May 1981, the nuclear establishment was worried. His socialist party, a relatively new and unknown force in French politics, had committed itself to a fairly strong anti-nuclear policy. Paul Quilès, the architect of the socialists' plan, was effectively seeking a halt to the construction of new nuclear power plants. If this plan had been implemented, the monopoly electricity supplier (Electricité de France, EDF) would have had only 39 GW of nuclear electricity available in 1990, compared with the 59 GW planned by the previous government, and EDF's supplier of nuclear steam supply systems — Framatome — would have found itself with (to say the least) an embarrassing overcapacity. With the export market also sluggish, this would probably have meant the collapse of the French nuclear industry.

However, six days after the presidential election, a strange thing happened. Giscard d'Estaing's centre-right government was in its last few days of limbo, before the elections to the National Assembly (parliament)

and the establishment of the new government. Usually, no major decisions or commitments are taken at such times. But Raymond Barre, Giscard's Prime Minister, unexpectedly announced that the reprocessing facility for spent nuclear fuel at Cap de la Hague, near Cherbourg, was to be massively extended — at an estimated cost of FF 20,000 million (£2,000 million) — to cope with 1,600 tonnes of fuel a year. Some of this capacity was to honour contracts already signed with Belgium, Germany and Japan; but the rest implied a strong national nuclear programme. The decision could hardly have been taken without the tacit agreement of Mitterrand, who would have to carry the policy through. So what exactly was the new government's position on nuclear power?

Initially, it seemed as if the Quilès policy might be adhered to. One of the Mitterrand government's first acts was to "freeze" construction on five nuclear sites — Chooz, in the Ardennes near the Belgian border, Cattenom, Civaux, Golfech near the Pyrénées and Le Pellerin. Plans for a nuclear plant at Plogoff in Brittany were

shelved, at least temporarily.

But then other notes were sounded. Jean-Pierre Chevènement, the minister of state for science and technology and a powerful figure in the new government, declared himself solidly behind the French nuclear programme, and all but described the small French environmental movement as "anti-scientific".

Discarding the chaff

Speaking cynically, it is as if Mitterrand had used the environmentalist tendency in his socialist party only so far as was necessary to help build the party and gain power. Now it was possible to let such chaff blow away. The environmentalists do feel themselves hard done by. Giscard had not even lent them an ear. Mitterrand had appeared to listen, and then ignored them. Recently there was a rocket attack on Superphénix, the world's first commercial-scale fast breeder power station. Nobody has claimed responsibility, but there are fears at EDF that this means that an extreme section of the environmental movement has now gone underground.

Although the Mitterrand government can in no sense be called "green", it is showing itself to be sensitive to arguments for more democratic control of technical choice. Mitterrand is emerging as a social democrat, although he would not dare to use this label within his party.

In the energy sphere, the evidence for the socialist government's liberalism came first with an energy debate in the National Assembly last October. In contrast to the Quilès policy, the energy policy revealed by Prime Minister Pierre Mauroy and the junior minister for energy, Edmond Hervé, was very mild. It was forced through against the Quilès faction on a vote of confidence. Instead of 59 GW nuclear in 1990 there would be 56 GW. Instead of nine new reactor starts in 1982-83, there would be six. The La Hague development would go ahead. The fast breeder was not debated. The frozen reactor construction sites would be reopened, if a local vote approved; if it did not, it would be up to the regional council (a higher elected authority) to decide; if the regional council did not approve, the matter would come before the National Assembly — a mechanism designed, it seemed, to get a "yes" vote at some level or other.

The environmentalist lobby looked on the black side — this was just the old centralist technocracy by another name. But there is another view, which as time passes comes more and more to the fore. The Mitterrand government is fiercely realistic — while at the same time wanting to make historic and lasting changes in the nature of French society. It is not going to compromise either on economic development or on a slow, emphatic shift of power away from the Paris-based élite, towards the regions, towards the small enterprises rather than the giant conglomerates.

Realism leads the government to under-

Media plug in here

JUST to the side of the Electricité de France (EDF) edifice in Paris is an unobtrusive set of doors. In the case of a major nuclear accident at one of the EDF power stations it is through this door that most of the world's (official) information about it will come.

For behind this door is a FF 4-million (£400,000) communications suite, the brain-child and pride of EDF's chief public relations officer, Mme Marie-Claude Vigna. Mme Vigna began to plan the suite after a French power black-out in 1979 had led to a great press of journalists in a tiny room in EDF headquarters, all attempting to use five telephones at once. If it was like that for a mere power breakdown, what about a "Three Mile Island", Mme Vigna reasoned?

The consequence is that EDF now has probably the most sophisticated "press office" in France. At street level, television vans can simply plug in to a series of channels giving television output from the suite above. Within the suite, sound-insulated rooms take tapes direct from the major wire services such as

Agence France Presse and Reuters, so that journalists — and EDF staff — can monitor media output by the minute. There are of course more than five telephones; a television studio, executive briefing room; and literally every known form of video recording and playback equipment. And officials being interviewed can be relayed information over private monitor screens which are within their vision but not that of the journalists interviewing them (see photograph below).

Outside Paris, hundreds of EDF officials throughout France will be kept in touch by a private videotext signal, sent through the French ANTIOPE system.

A French prefect (regional head man) who was responsible for the region around the Dampierre nuclear power station recently sent out a notice to his constituents telling them what to do in case of a nuclear accident. Don't panic, he said. Keep your children indoors. Don't eat apples from the garden. And, mysteriously, don't telephone. Well, there'd be no need to with what EDF has in Paris, would there!

Energy minister Edmond Hervé confronts the press in EDF's media room



stand that a country 70 per cent dependent on foreign fuel, whose healthy non-oil trade balance is wrecked by the cost of oil imports (99 per cent of oil is imported), must attempt to internalize the costs of energy production. Nuclear electricity is part of the solution. The government is also realistic enough to know that the wholesale destruction of the nuclear industry would be immensely demoralizing to French people — who, while being as suspicious of nuclear power as the next nation, see its success as one of the great symbols of French strength. France has been overrun by a foreign power three times since the Revolution, and no Frenchman is going to let it happen again — either militarily or economically.

Handling the public

On the other side of the equation, the small group of men at the head of EDF, the CEA and Framatome, who effectively control the nuclear development of France, are gradually being forced to pay serious attention to the general concerns of the public and the particular demands of the regions

where nuclear facilities are being sited.

For example, when the government says it will "reinforce the independence" of the Conseil Supérieur de la Sécurité Nucléaire (the senior nuclear safety council) and "modify its composition", it means that it really will attempt to detach the safety council from its tutelage to the nuclear establishment. The proposal to create a "safety director" under the control of EDF may be looked at a little askance, as may guarantees of the independence of the Institut de Protection et de Sécurité Nucléaire, the technical safety assessment body which exists under and has constant exchange of staff with the Commissariat à l'Energie Atomique; but the establishment of local information commissions on each nuclear site could be taken more seriously.

These commissions are proving slow to set up (so far there is one at La Hague and another at Nogent-sur-Seine) but, according to commitments made by the government in the energy debate, they will be pluralist, "contradictoire" (in other words allowing serious debate and close questioning), independent and permanent.

Hard bargaining at Golfech

The apparently anodyne decision to subject the five frozen construction sites to a hierarchy of democratic assessments, from local to national level, until at some stage somebody said "yes", has also proved a thorn in the EDF flesh. The votes went in favour of continuing construction at Chooz, Cattenom and Civaux. But at Golfech, near the border with Spain, and Le Pellerin, there were marginal local votes against. So the question went to a higher level — the regional council. The Le Pellerin council voted for the reactor. But Golfech, while eventually agreeing, drew blood from EDF in the process.

The "blood" took the form of a contract with EDF — the first ever such binding arrangement between EDF and a region — which guarantees the locality 40 per cent of the jobs that will eventually be created at



Michel Rapin of CEA says the nuclear programme is stronger under Mitterrand

the power station, a large fraction of the construction work and EDF support for amenity development. EDF is currently seeking sites for five reactors at 1,300 MV and one at 900 MW — the programme agreed at the National Assembly for 1982–83 — and the Golfech deal may prove to be an awkward model. EDF, however, argues that it has always tried to place jobs and contracts around the region of a nuclear site. The real difficulty, says EDF, will be to find 40 per cent of its nuclear engineers in the Midi-Pyrénées.

The latest twist in the tail is literally there — at the tail end of the nuclear fuel cycle. Waste disposal has been neglected in France (although the Marcoule process for the vitrification of highly active waste has been adopted by Britain). Edmond Hervé, minister for energy, has asked the CEA to provide, within a few weeks, an outline plan for the management of nuclear wastes. No such plan exists at present, it seems. Within 18 months, two or three disposal sites must be selected, promising a new political problem for nuclear power.

Some in the nuclear establishment, however, are quite sanguine about the developments. Michel Rapin, director for nuclear applications at the commissariat, believes the new political approach will actually strengthen the nuclear programme, by providing a degree of democratic assessment. Once there has been a vote, who can disagree? All very well, while the votes are "yes". . . □

Trebling renewable energy by 1990

ONE of the most surprising but rational moves made by the Mitterrand government in the energy field has been its appointment of Michel Rolant, a union activist in his late 40s, as the head of a completely revamped organization to promote renewable energies in France.

Rolant was an agricultural worker who took a strong interest in labour relations, and early on became general secretary of the federation of agricultural workers, a branch of the liberal union, CFDT. But his attention shifted to industry and employment, and he established a reputation, among the pro-nuclear lobby, of being totally anti-nuclear. Certainly, as CFDT's number two, Rolant moulded the union to form effectively the only organized opposition to the nuclear establishment — a position which it will retain despite Rolant's departure.

Electricité de France is turning a rather jaundiced eye on Rolant's appointment — the cosy club of grandes écoles men at the top of the ministries and the great industries is being jostled these days by one or two workers with some peculiar ideas — but for the government the move is really a master-stroke (and, by the way, one long advocated by CFDT). For, from the nuclear point of view, one of the principal irritants has been mollified: he

to be determined. The CFDT recommends a balanced regional and central organization, with 22 regional branches each with perhaps 50–100 staff, and a 600–700 strong national body. (By comparison, COMES and AEE presently have about 500 staff mostly in Paris). The new body should also be capable of doing research on its own behalf, together with the major research organizations.

Moreover if capital loans could be arranged on the kind of terms on which they are offered to Third World nuclear purchasers — terms such as 8 per cent interest to begin five years after completion of a reactor as were offered to Korea, for example — then wonders could be worked, say the enthusiasts.

In fact COMES announced an objective at the end of last year: to treble the funds devoted to solar energy between 1981 and 1985. The most promising areas for development were the production of petrol and methane from biomass, particularly wood waste of which France has a particularly good supply. But Rolant will want to see all alternatives, including energy saving, in perspective, and perhaps COMES's priorities will not be his. The government is certainly committed in principle to a trebling of funds for renewable energy projects, although the allocation of the

Consumption of primary energy: evolution and objectives*

	1970	1974	1980	1981	Plan 1990
Coal	38.1	31.6	34.0	33.5	35–40
Petrol	87.5	113.2	102.1	93.0	70–75
Gas	9.3	16.0	23.6	24.6	31–40
Hydroelectrics	12.4	12.5	16.0	15.0	14–15
New energies	2.0	2.1	3.2	3.4	10–14
Nuclear	1.2	3.1	12.9	19.5	60–66
Total	150.5	178.5	191.8	189	232
Proportion from indigenous sources (%)	65.6	75.0	71.0	68.0	45–50

*Values are in million tonnes of oil equivalent

is now part of the government club and must be expected to obey the rules. And from the alternative energy point of view, here is a man who is a passionate and serious advocate of such forms of energy who must now turn his dreams into reality. If they work, well, that means more energy for France.

In fact the government's energy plans for the next decade are extraordinarily ambitious both in respect of their nuclear component and their renewables (see table). The energy supply from renewables — such as solar power and biomass — is expected at least to treble by 1990 compared with 1981, and M. Rolant will be presiding over that growth. Rolant will have available to him COMES, the commission for solar energy, and the AEE, the agency for energy saving, which will be formed into a new organization whose exact definition and scale have yet

cash will not be known until mid-1982.

No doubt Rolant will have considerable influence on this, and perhaps his agricultural background will influence him (his colleagues at CFDT deny it); but it has been clear for some time that in renewables France is concentrating on the conversion of biomass. Of the 10–14 million tonnes of oil equivalent expected from renewables in 1990, half will come directly from the use of wood, and a further 2 million tonnes of oil equivalent from wastes. Surprisingly, however, of FF 35.2 million (£3.5million) research ministry funds available for renewables research in 1981, only FF 0.2 million (£20,000) went to support biomass research. The largest sum (around half) went to coal. COMES spent FF 50 million (£5 million) on biomass development in 1981, but only a small fraction of that went on basic research. This may change.

NEWS AND VIEWS

Demon nuclei— spurious excitement?

from Frank Close

CAN quarks, the hypothetical ultimate constituents of all nuclear matter, cluster together and form 'demon' nuclei — an exotic form of nuclear matter in which individual protons and neutrons cannot be discerned? The existence of such matter has been recognized as a possibility for some time, but now great excitement has been caused by a claim that 'demon' nuclei may actually have been detected. Early this year S. Fredriksson and M. Jandl of the Royal Institute of Technology in Stockholm (*Phys. Rev. Lett.* **48**, 14; 1982) predicted, from a new theoretical approach, that demon deuterons might have been responsible for anomalies seen both in cosmic experiments and in recent experiments at the Lawrence Berkley Laboratory. However, an even more recent paper suggests that the excitement may be premature. H. J. Lipkin of the Weizmann Institute (*WIS Report* 82/2, January; 1982) raises powerful theoretical objections to the existence of demon deuterons and shows that whatever is responsible for the experimental anomalies, it cannot be demon nuclei.

The ways in which quarks cluster together to build up nuclei can be thought of as similar to the ways that electric charges cluster together to build atoms and molecules. In the latter case, electromagnetic attraction between opposite charges causes electrons to encircle the atomic nucleus to build up atoms whose total electrical charge vanishes. Neighbouring atoms are held together by three varieties of forces, each of which has its origin in the fundamental electromagnetic force. Neighbouring atoms with an excess or deficiency of an electron can be directly attracted (ionic force); electrons can be shared among atoms (covalent force); or there can be an asymmetric distribution of charge within the overall neutral pattern (van der Waals' forces).

Quarks carry a property called colour which is similar to electrical charge except that instead of there being just one variety of positive charge there are three varieties (red, yellow and blue) of positive or negative colour. Unlike colours attract just as unlike charges do. Quarks carry positive

colours and their antimatter equivalent, anti-quarks, carry the negative or complementary 'anticolours'. Quarks and anti-quarks will thus be mutually attracted and form the variety of matter known as mesons, of which the pion is the most familiar example. However, because of the threefold nature of the colour charge there are possibilities for quark clusterings which do not have a direct analogue in electromagnetism.

For example, two 'red' quarks will repel one another, but if a red and yellow one are nearby they will be mutually attracted. If a third quark carrying a blue colour is near to this pair, then the red and yellow, yellow and blue, and red and blue will all mutually attract one another and can form a stable system known as a baryon, of which the neutron and proton are the lightest examples. If a fourth quark comes near to this system it will be repelled by the red-, yellow- or blue-coloured quarks depending on which colour it is itself carrying. Three quark clusters are thus very stable and this is the source of the baryon's existence.

In the cluster of red, yellow and blue quarks that forms the baryon, the total colour has cancelled out leaving no net colour; just as the electrically neutral atom results from electromagnetic forces. In turn, the forces that cluster adjacent neutrons and protons to one another to form nuclei are the colour analogues of the covalent or van der Waals' forces of electromagnetism. An important difference between colour forces and electromagnetic forces is that colour ions appear to be forbidden to exist as free entities in nature. Thus individual coloured quarks or pairs of quarks which carry net colour have no free existence, although this need not prevent the existence of colour ions within densely packed clusters — so long as the total colour of the whole cluster vanishes. Indeed, within the proton there exist two quarks with net colour which are attracted to the third quark which itself

carries colour; in this sense the proton is built of a di-quark coloured ion and a quark coloured ion. Could there, then, be ionic forces between di-quarks within nuclear matter? The simplest example would be in a cluster of six quarks of which the most familiar example is the deuteron; three quarks form an uncoloured cluster known as the proton and the remaining three form an uncoloured cluster, the neutron. The neutron and proton then attract by the residual colour forces, analogous to covalent forces (pion exchange) or to van der Waals' forces (about which there is much theoretical discussion but not total consensus yet). However, there is also the possibility that this total of six quarks, instead of forming two uncoloured clusters of three, might form three colour ions of two! Thus the deuteron, which is two sets of 'tri-quarks', could have a demon brother which is a set of three di-quarks. The di-quarks carry colour and will be strongly attracted to one another, in a manner analogous to ionic forces. They will be bound very tightly in a cluster of six quarks, in contrast to clustering into groups of three where the residual force would be expected to be weaker. Thus, it seems in principle possible that a stable cluster of three di-quarks exists in nature, perhaps lighter and more stable than the familiar deuteron.

This idea is at the root of the paper by Fredriksson and Jandl. The authors predict that their demon deuteron will be light enough to be almost stable and might be responsible for the anomalous nuclear interactions that have been seen in cosmic experiments (the Judeck effect) and in recent experiments at the Lawrence Berkley Laboratory (E.M. Friedlander *et al.* *Phys. Rev. Lett.* **45**, 1084; 1980). When a high-energy nucleus collides with an emulsion target, it was discovered that about six per cent of the fragments created in the collisions travelled only very short distances, much less than normal, suggesting that they were somehow anomalous with very high reaction cross-sections, long lifetimes and stable against strong decays. Fredriksson and Jandl suggest that the large interaction cross-section arises

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because 'demon nuclei' are produced.

Even more recently, important difficulties with this suggestion have been raised by H.J. Lipkin of the Weizmann Institute. Crucial in creating a demon deuteron from three di-quarks is the role of the Pauli principle. Having all three di-quarks in s orbitals violates the Pauli principle and so the authors suggest each di-quark is in a p orbital, the three of them coupling to a total $L = 0$ state with negative parity.

Lipkin points out that as there are only two independent orbital angular moments in a three-body system, then the $J^P = 0^-$ object made from three $J^P = 0^+$ di-quarks cannot exist. The so-called demon state is a spurious state of centre-of-mass motion in which the centre-of-mass of the three di-quarks is oscillating in a p-wave. When this

spurious excitation is removed by putting the centre-of-mass in an S-state then one obtains an allowed state with total angular momentum of one. This isoscalar $J^P = 1^+$ has the same quantum numbers as the deuteron, might very well be mixed into the deuteron's wave function and be detectable in measurements of the deuteron's form factors. However it would not be expected to occur as a separate metastable state.

Thus the observation of anomalous nuclear processes on the one hand and the possible occurrence of new multi-quark configurations in nuclear matter on the other remain, for the moment, unconnected. If demon nuclei do exist it seems that they do not occur in the way that the Swedish group claim. □

noted the resemblance of many features of the skull and skeleton of the thecodontian *Chasmatosaurus* (*Proterosuchus*) to those of rhynchosaurs. Carroll^{4,5} developed these ideas further and demonstrated close similarities between *Heleosaurus*, a late Permian eosuchian, and the early Triassic thecodontian *Euparkeria*. Although clearly similar to *Youngina*, *Heleosaurus* had a dentition very like that of *Euparkeria* and it may have been capable of an upright posture, an advanced feature of many archosaurs. Gow⁶ redescribed *Youngina*, and also suggested that it was close to the ancestry of archosaurs.

New information on the affinities of rhynchosaurs and sphenodontids is also damaging to the integrity of Romer's Lepidosauria for it is believed that these two groups are particularly closely related. Carroll⁷ redescribed *Noteosuchus*, a partial skeleton from the early Triassic of South Africa, and interpreted it as the oldest known rhynchosaur. Its ankle structure is similar to that of *Chasmatosaurus* (*Proterosuchus*). Carroll stressed that there is no evidence for a close relationship between rhynchosaurs and sphenodontids and that supposed shared characters are either primitive features of all diapsids, or they have been wrongly interpreted. For example, the living *Sphenodon* has acrodont teeth (fused to the summit of the jaw bone), while rhynchosaurs had deeply rooted teeth. *Sphenodon* has a row of teeth on two

The Diapsida: revolution in reptile relationships

from Michael J. Benton

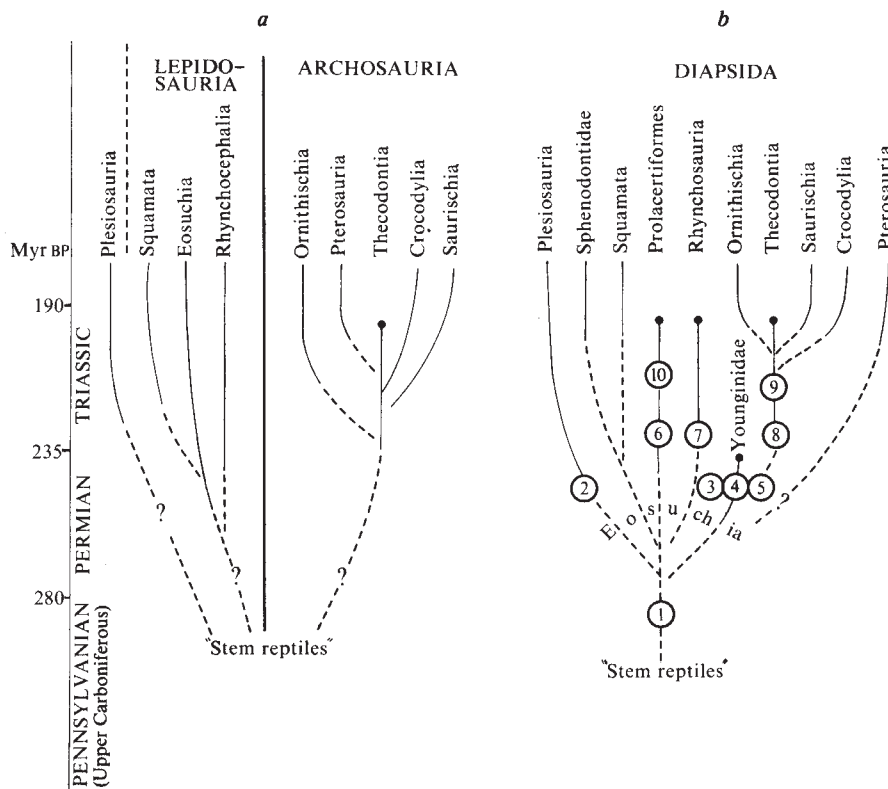
DISCOVERIES of new fossils and reinterpretation of existing data suggest the need for a major change in the traditional view of the relationships and ancestry of the lizards, snakes, crocodiles, dinosaurs and many extinct groups of reptiles. In standard textbooks (for example, Romer's *Vertebrate Paleontology*), the reptiles are divided into subclasses according to the number of openings behind the eye sockets. Those with two openings, the Diapsida, are further divided into two groups, the Lepidosauria (including lizards, snakes, sphenodontids, rhynchosaurs and eosuchians) and the Archosauria (including the thecodontians, crocodiles, dinosaurs and pterosaurs), that are thought to have diverged from separate ancestors as early as the Upper Carboniferous (Pennsylvanian; see Fig. 1a). The new view (summarized in Fig. 1b) is that all diapsids have a common ancestry and that their classification will have to be completely revised.

Romer¹ considered that lepidosaur groups, including the lizards, snakes, sphenodontids and rhynchosaurs (medium-sized 'beaked' and probably herbivorous reptiles of the Triassic), all derived from the eosuchians, a mixed group of primitive forms, of which *Youngina* from the late Permian of South Africa is usually regarded as typical. In contrast, archosaur groups, including the crocodiles, dinosaurs and pterosaurs, were considered to be derived from thecodon-

tians, such as the early Triassic *Chasmatosaurus* (*Proterosuchus*).

More recent results now suggest that both lepidosaurs and archosaurs had common ancestors among the eosuchians^{2,3}. Cruickshank³ particularly

Fig. 1 Evolution of some major groups of reptiles, according to Romer¹ (a) and to recent work (b). Animals mentioned in the text are shown in b as follows: 1, *Petrolacosaurus*; 2, *Claudiosaurus*; 3, *Youngina*; 4, *Thadeosaurus*; 5, *Heleosaurus*; 6, *Prolacerta*; 7, *Noteosuchus*; 8, *Chasmatosaurus* (*Proterosuchus*); 9, *Euparkeria*; and 10, *Tanystropheus*.



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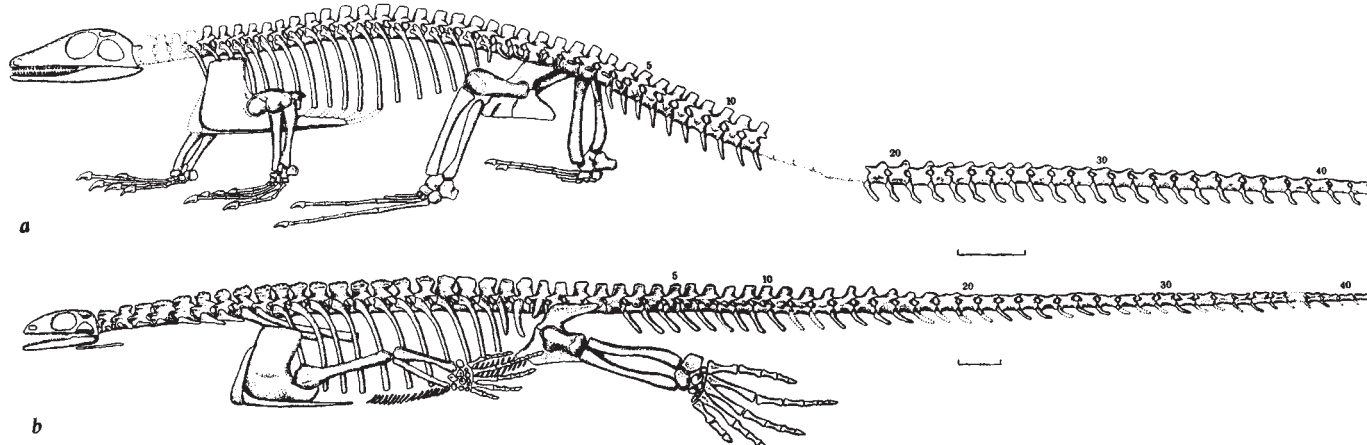


Fig. 2 Restorations of the skeletons of *Thadeosaurus* (a) and *Claudiosaurus* (b) in side view. *Claudiosaurus* is shown in a swimming pose. Scale bars are 2 cm. The similarities between *Claudiosaurus*, supposedly the earliest plesiosaur, and *Thadeosaurus*, a contemporary eosuchian from Madagascar, suggest a close relationship. After Carroll¹⁴.

separate bones of the palate (the maxilla and the palatine), while the multiple rows of teeth in rhynchosaurs are all on the maxilla. *Sphenodon* appears to show more affinity with ancestral lizards.

New evidence also suggests that the Pterosauria should be removed from the Archosauria and recognized as a distinct group. As early as the late Triassic, pterosaurs from Italy, described by Wild⁸, had all the special adaptations of the group for flight and had diversified into two quite different stocks. Wild suggested that pterosaurs originated directly from the eosuchians in the early Triassic since their anatomy suggests that they derived from small running insectivorous forms. The thecodontians of the Triassic were generally too large or otherwise unsuitable as ancestors, and there would probably have been insufficient time for a radiation from them to have occurred.

We can now discern four separate lineages of diapsid reptiles that radiated from the Eosuchia in the late Permian: pterosaurs, thecodontians, rhynchosaurs, and a group made up of lizards, snakes and perhaps sphenodontids. But how do we define the Eosuchia?

Romer¹ included a broad group of Younginiformes, the Prolacertiformes (centred on *Prolacerta*, an advanced form from the early Triassic of South Africa, that shows some parallel features, to lizards) as well as some later aquatic forms. Evans⁹ noted that the Eosuchia have no diagnostic advanced characters and can only be defined by the absence of features typical of other groups. Thus, she broadened the Eosuchia to include all diapsid reptiles except the archosaurs, the lizards and snakes. Further work may enable us to extract some of the motley collection of reptile families from the eosuchian rag-bag when we understand more about the diapsid radiations in the Permian and Triassic.

The confusion over the definition and limits of the Eosuchia makes it difficult to decide whether the Eosuchia had a

common ancestor, but an apparently suitable form exists. *Petrolacosaurus* from the late Pennsylvanian (260 million years ago) of Kansas has been redescribed recently on the basis of much new material¹⁰. It was clearly a diapsid, but certain features also indicate its ancestry among the 'stem reptiles'. Chatterjee¹¹ has included *Petrolacosaurus* in the Prolacertiformes, together with several Permian and Triassic reptiles. He regards the enlarged prolacertiform group as a lineage evolving separately from other eosuchians. Wild¹² has independently come to a similar conclusion on the basis of a re-study of the curious long-necked *Tanystropheus* from the middle Triassic

period of Switzerland.

One recent study adds a further dimension to the diapsid reptiles by suggesting that the aquatic plesiosaurs, previously of uncertain affinities, derived from the Eosuchia. Carroll¹³ described two reptiles from the late Permian of Madagascar: *Thadeosaurus*, a younginid eosuchian, and *Claudiosaurus*, which he interprets as the first plesiosaur (see Figs 2,3). *Claudiosaurus* resembles younginids in its general anatomy, but it lacks the lower temporal bar and has a closed palate — typical plesiosaur features. Although it does not show all of the particular adaptations for aquatic locomotion that plesiosaurs had (paddle-like limbs, streamlined body, strong tail), certain features suggest that it was a swimmer (poorly ossified wrist and ankle, proportions of the limbs, small skull, long neck). The ancestry of plesiosaurs has been problematical, but Carroll makes a strong case for their derivation directly from diapsid reptiles during the Permian.

There now seems to be little evidence for the separate status of the Lepidosauria and the Archosauria, and it appears that all diapsid reptiles can be derived from *Petrolacosaurus*. Thus, we may re-instate the Subclass Diapsida, established by Osborn¹⁴ in 1903. This group will now contain a large number of forms, many of them poorly known, and future work must concentrate on sorting out their relationships. □

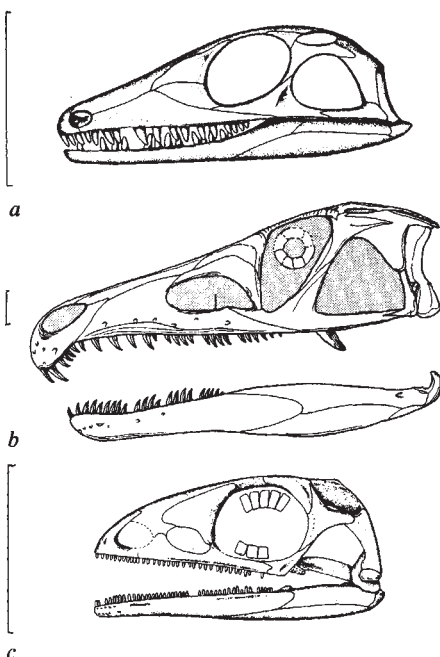


Fig. 3 Side views of the skulls of *Youngina* (a), *Chasmatosaurus* (*Proterosuchus*) (b) and *Claudiosaurus* (c), an eosuchian, a thecodontian and what may be the earliest plesiosaur respectively. These early representatives of the groups show considerable similarity. Scale bars are 2 cm. After Carroll¹⁴ and Cruickshank³.

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2. Hughes *J. Zool., Lond.* **156**, 457 (1968).
3. Cruickshank *Studies in Vertebrate Evolution*, 89 (Oliver & Boyd, 1982).
4. Carroll *Colloque int. C.N.R.S.* **218**, 433 (1975).
5. Carroll *Athlon*, 58 (Royal Ontario Museum, 1976).
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7. Carroll *Ann. S. Afr. Mus.* **72**, 37 (1976).
8. Wild *Bull. Soc. paleont. Italiana* **17**, 176 (1978), and see Cox, B. *Nature, News and Views* **284**, 400 (1980).
9. Evans *Zool. J. Linn. Soc.* **70**, 203 (1980).
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12. Wild *Schweiz. paläont. Abh.* **102**, 1 (1980).
13. Carroll *Phil. Trans. R. Soc. B* **293**, 315 (1981).
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Two bar mitzvahs for tubulin

from Chandler Fulton

AMONG the thirteen years since tubulin was named¹, the past year has been particularly exciting*. Complete sequences have been reported for both α -tubulin and β -tubulin subunits, the heterodimer of which constitutes tubulin, and it is now clear that cells contain multiple tubulins encoded in multiple genes.

Tubulin is the major building block of microtubules. All eukaryotes have microtubules, so the first eukaryote

presumably had tubulin, probably already utilized for the conserved functions of mitosis and flagellar movement. Yet the origin of tubulin is lost in antiquity (about 3×10^9 BC). Margulis² has provocatively proposed that microtubular structures arose through integration of an endosymbiotic spirochete into a proto-eukaryote, but there is only tenuous evidence to support this view.

Three groups have reported the complete sequencing of tubulin subunits from brain, both by direct analysis of pig brain tubulin subunits^{3,4} and by sequencing of

cDNA clones to chick brain tubulin mRNA⁵ and rat brain α -tubulin mRNA⁶. One immediate consequence is that the molecular weight of both subunits is reduced from the widely quoted 55,000 to 50,000 (450–451 and 445 amino acid residues for α - and β -tubulin respectively). The complete sequences confirm earlier insight that the two subunits share considerable homology^{3–5}. They also support the view that tubulin is among the most conserved of proteins; in 270 million years of separate evolution leading to chickens and pigs, only about 7 of the 855 sequenced residues have been altered. The available information suggests that the subunits diverged from a common, unknown ancestor, and since then their evolution has been held in check, perhaps by functional constraints on the protein. Preliminary evidence based on peptide mapping suggests that protistan tubulins have α -subunits quite different from vertebrate tubulins⁷. It seems probable that the sequencing of additional tubulin subunits will shed light not only on the structure and function of tubulin but also on the evolution of eukaryotes.

The amino acid sequences and molecular cloning experiments both support the conclusion that many eukaryotes contain and express multiple tubulin genes to produce tubulins that differ slightly in primary structure, an hypothesis that seemed improbable to most when it was proposed six years ago. The protein sequences indicate at least four different α -subunits and two different β -subunits in pig brain tubulin^{3,4}. There are at least two α - and two β -tubulin genes in *Chlamydomonas*^{8,9}, four of each in *Drosophila* and chickens, and more than ten in sea urchins, rats and humans¹⁰. In *Drosophila* and in chickens, the multiple tubulin genes are dispersed in the genome (see, for example, ref. 11). All four α -tubulin genes are expressed in *Drosophila*¹², and E.Y. Lai and I have found that at least two β -tubulin genes are expressed during the differentiation of *Naegleria amoebae* to flagellates. Although the coding regions of tubulin genes are sufficiently conserved for complementarity to be detected among diverse eukaryotes, from yeast to humans⁵, the flanking regions are divergent even within a single organism (see, for example, ref. 12). The existence of multiple tubulins and tubulin genes requires revision of many of our ideas about tubulin, as well as re-interpretation of old experiments, especially those that measured tubulin pools and synthesis as if tubulin were a homogeneous protein. It also raises questions about the role of multiple tubulins, in particular whether the different tubulins are used selectively in building microtubular organelles. Actin too has been found to be a family of genes and proteins¹³, which raises similar issues for this protein.

Instructive tubulin mutants are beginning to appear. A testis-specific

*This report is inspired by two meetings which might be said to have celebrated tubulin's reaching, in human terms, the age of responsibility: a joint symposium of the Embryology and Physiology courses at the Marine Biological Laboratory on "Organization and Expression of the Genes for Tubulin", organized by R. Raff and J. Rosenbaum and held at Woods Hole on 17 July 1981, and a European Molecular Biology Organization Workshop, "Microtubules in Microorganisms", organized by P. Cappuccinelli and held at Porto Conte, Sardinia, on 8–12 September 1981.

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100 years ago

MAXIM'S SELF-ACTING FIRE-EXTINGUISHERS

HOWEVER certain it is that fires in theatres will never be completely suppressed, we may still hope by energetic measures and systematic arrangements to lessen both their number and their danger.

The stage is undoubtedly the most dangerous point, from the very nature of the materials composing it. M. Maxim's proposes to institute such a preventive system that, as soon as a fire begins to show itself at any given point of the stage, the accident will itself produce automatically and instantaneously a series of mechanical movements sufficient to flood the threatened part with water, and arrest the progress of the fire.

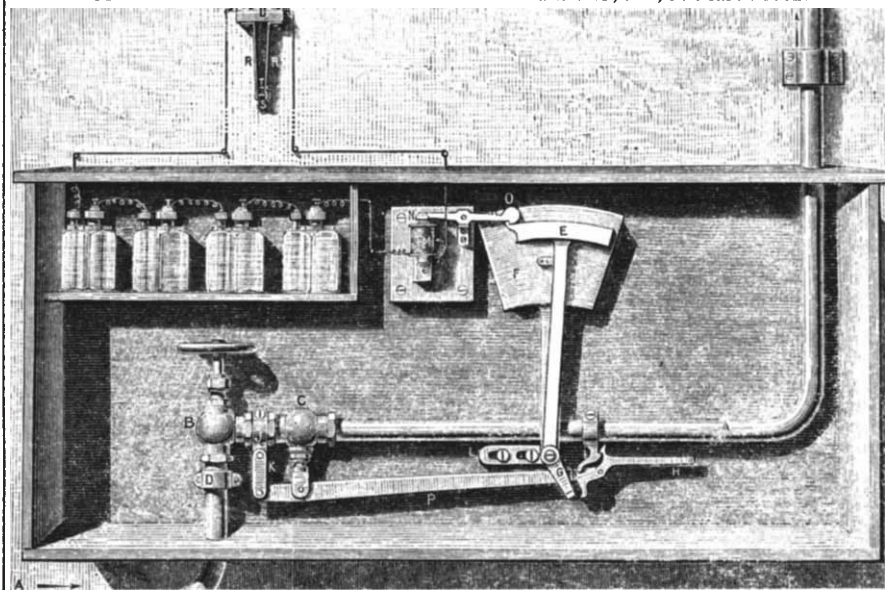
The self-acting *Electric System* he proposes is composed of three distinct parts: (1) an apparatus which completes the electric circuit, under the action of the raising of temperature caused by the fire; (2) a self-acting supply-cock, to send water into the system of pipes from the main pipe in the street; (3) an arrangement for opening the discharge-pipes upon any part which is in danger.

The apparatus (1) for completing the elec-

tric circuit is extremely simple. It is composed (Fig. 1) of two metallic plates, R, R, forming springs, and separated by a small piece of fusible metal, S, which is isolated from the plates by paper, or any other isolating body. The heat caused by the fire melts the metal, and the plates coming into contact, the electric acts upon the automatic supply-cock. This self-acting supply-cock (Fig. 1) is composed of an electro-magnet, M, which, under the action of the current, becomes active, and attracts the arm N, thus setting free the lever, E. The weight, F, then turns from right to left, and, after describing the quarter of a circle, falls upon the lever, H, whilst the part, G, removes a check which has kept the lever, P, in position. Under the action of the weight, F, and by means of the lever, P, which has its fulcrum in the point, J, the supply-cock, C, is turned, and the water rushes into the pipe, A, to be discharged above. The cock at B, which is worked by hand, serves to stop the supply when the fire is extinguished.

The water pouring upwards into the pipes ought to be discharged at the point where the fire appears. Ordinary perforated pipes may be used, and the system of pipes may be disposed around the stage.

From *Nature* 25, 511, 30 March 1882.



β -tubulin mutation has been described in *Drosophila*¹⁴, which provides evidence that there is more than one functional β -tubulin in this organism. Oakley and Morris¹⁵ have carried out ingenious work on benomyl-resistant mutants of *Aspergillus*. A class of these mutants was shown to contain mutations of a β -tubulin structural gene (*benA*). Some *benA* mutations alter the isoelectric point of β -tubulin, and these always alter the mobility of two β -tubulin isoforms; thus both isoforms are products of the *benA* gene. Revertants of a *benA* mutation were used to isolate a mutation in an α -tubulin gene. A temperature-sensitive *benA* mutation, with hyperstable microtubules, was used to show that, as Inoué has long argued, microtubule depolymerization is involved in chromosome movement.

The most-studied tubulins have been isolated from vertebrate brains, which are rich in the protein, by cycles of assembly into microtubules and disassembly, but until recently attempts to isolate tubulin from the cytoplasm of protists have been unsuccessful. The systematic isolation of polymerizable tubulin from *Physarum amoebae*¹⁶ and from yeast¹⁷ should encourage future attempts and provide opportunities for much-needed comparative studies. Although many properties of tubulin and microtubules, from antigenic determinants to ultrastructure, have been conserved during the evolution of eukaryotes, others seem to vary. For example, whereas colchicine inhibits the assembly of microtubules in diverse eukaryotes, it has little effect on the polymerization of tubulin in many protists. In some of these protists polymerization of tubulin is quite sensitive to benomyl^{16,17}. Although we continue to learn from the exhaustive studies of brain tubulin, novel studies of other tubulins are likely to change our perspective.

The cylindrical walls of microtubules usually contain 13 protofilaments (strings of tubulin), but many exceptions have been reported. Kilmartin found that when

yeast tubulin is polymerized *in vitro*, in the first assembly there are 12 protofilaments, whereas there are 13 in the second cycle¹⁷. In *Caenorhabditis*, Chalfie¹⁸ found that although most of the neuronal microtubules have only 11 protofilaments, six touch-receptor cells have microtubules containing 15 protofilaments. Experiments using mutants, colchicine and benomyl indicate that these 15-protofilament microtubules are important for sensory transduction. Proteins associated with microtubules, of which many are known, may determine the number of protofilaments, but we have little understanding of this process. The temporal and spatial orchestration of the

intracellular assembly of tubulin into precise arrays of microtubules remains even more mysterious, although elegant descriptive studies of these arrays are beginning to address the issue (for examples, see ref. 19).

In the few years that tubulin has been studied, we have just become acquainted with the possibilities explored during the evolution of cells that utilize this conserved structural protein in cell shape and motility. Much has been learned, but how long will it take us to understand the important features of a crucial eukaryotic protein that has been experimented with for billions of years, among countless billions of organisms? □

Was Precambrian seawater different?

from Peter J. Smith

MINERALOGICALLY, dolomite is a simple carbonate of calcium and magnesium. As far as its genesis is concerned, however, it is rather more of a puzzle. Although common in Precambrian rocks, dolomite is apparently no longer being laid down, except in a few marginal environments such as supralittoral zones and ephemeral lakes, even though seawater is supersaturated with it and, on thermodynamic grounds, it should be precipitated in preference to calcite or aragonite (forms of calcium carbonate). Moreover, when it has formed in recent (that is, Phanerozoic) times, it has usually been of the replacement type, which means that it began as calcite or aragonite and later had part of its calcium replaced by magnesium. The geological consensus is that the amount of primary dolomite precipitated directly from seawater during the Phanerozoic has been insignificant compared with the quantity of replacement dolomite generated; and observation shows that, over the same time span, the dominant primary precipitate from seawater has been calcium carbonate (limestone).

This modern preference for limestone has led many, if not most, sedimentologists to suppose that the same bias has always held. The preponderance of dolomite rock (dolostone) over limestone during the Precambrian is then readily explained in replacement terms; limestone has always been the preferential primary product but, being older, the Precambrian representatives have had longer to make contact with magnesium-rich, dolomitizing fluids. There is, however, at least one other possibility — that dolomite was itself the principal carbonate precipitate during the Precambrian because Precambrian

seawater was chemically different from its Phanerozoic successor. In that case, Precambrian dolomites (chiefly primary) and Phanerozoic dolomites (chiefly replacement) should be different in terms of their textures, fabrics and isotopic ratios.

That they can indeed be different has now been demonstrated by Tucker (*Geology* 10, 7; 1982). The Beck Spring Dolomite, which now outcrops in eastern California, is thought to have been laid down 1,200–900 million years ago in various environments ranging from subtidal shoals through lagoons to tidal flats. To Tucker, however, its most conspicuous feature, which it shares with many other Precambrian dolostones, is the extent to which its sedimentary structures have managed to preserve their detail on both broad and microscopic scales. There is no clear evidence of the destruction of original texture and fabric, nor is there any sign of subsequently imposed replacement fabric. Indeed, if other types of analysis had not shown the carbonate to be dolomite of Precambrian age, the Beck Spring Dolomite could well have been taken for an undolomitized Phanerozoic limestone. This is not to say that there have been no changes at all, for there is some evidence of neomorphism. But neomorphism does not necessarily mean mineral change; it could simply have involved wet recrystallization of dolomite.

On the basis of petrographical examination, then, the simplest interpretation is that the dolomite is primary and not a calcium carbonate replacement — a view that receives more than adequate support from isotopic data. Using a scalpel and a dentist's drill, Tucker went on to extract from the Beck Spring Dolomite samples of the various components (micrite, pisolites, fibrous dolomite, internal sediment and so on), which were

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then subjected to carbon and oxygen isotope ratio measurements using standard procedures. The results were, of course, numerical in detail, but the general picture is easily sketched. The data revealed distinct isotopic trends with all examples of each component falling within a distinct and limited field.

That such systematic information can be obtained at all emphasizes the importance of taking an inhomogeneous rock apart and analysing the components separately. Failure to do this in the past has often produced fuzzy data with large variations liable to misinterpretation. That such systematic information was actually obtained in this case, however, indicates that no major re-equilibration has occurred since the dolomite was laid down during the Precambrian. Since that time there has been no extensive interaction with meteoric waters, no deep burial, no more than minimal metamorphism and little deformation.

All the indications are, therefore, that the isotopic ratios and trends are original. What's more, those ratios and trends bear a striking similarity to those from primary Phanerozoic limestones. Tucker thus concludes that the Beck Spring Dolomite and, by implication, the many other Precambrian dolomite rocks with comparable properties were precipitated directly as dolomites and thus not generated by the dolomitization of directly precipitated limestones. And as the most important control on carbonates is the isotopic composition of the water from which they are precipitated, the inevitable, or at least the simplest, overall conclusion is that the Precambrian seawater that favoured the production of Ca-Mg carbonates (dolomites) must have been chemically different from the Phanerozoic seawater that gave, and gives, rise to Ca carbonates (limestones).

Unfortunately, Tucker does not elaborate on the difference, except to note the general consensus that the seawater characteristics favouring dolomite precipitation would include a higher than usual Mg/Ca ratio, a higher CO_2 partial pressure, a higher temperature and a lower SO_4^{2-} content. So mysteries remain. Why does dolomite not precipitate from modern seawater if, as Tucker claims, it is favoured thermodynamically? Why have experiments to synthesize dolomite under simulated sedimentary conditions merely resulted in an imperfectly ordered, Ca-rich version of dolomite ('proto-dolomite')? Why, if dolomite was the preferred precipitate during the Precambrian, were limestones nevertheless sometimes generated? And when (and why) precisely did the preference change from dolomite to limestone?

What the answers to these questions might say about dolomite is of comparatively minor importance, but what they might say about the evolution of seawater composition could be a rather bigger issue. □

Intermediate valence compounds as applicable materials?

from Michael Kelly

UNDER modest pressures, the normally semiconducting samarium sulphide undergoes a first-order phase transition to a metallic state. Thus by rubbing or scribing the surface of this material, an intrinsic metal-semiconductor contact can be generated and can subsequently be removed by temperature excursions of approximately 100°C .

Possible applications of this and other surprising properties of the so-called intermediate valence compounds, especially those involving compounds and alloys of the rare-earth elements, were outlined by P. Wachter (Zurich) in the closing session of a one-day meeting* devoted to the physical properties of these materials. As the name implies, the ionic constituents of intermediate valence compounds may carry non-integral charges. Briefly, in the solid environment, only a small energy difference may be involved in the transfer of an electron from an incomplete f shell of a rare-earth atom to the available states of the valence band. The result is that there may be dramatic anomalies of physical properties not so far explained by conventional valence band theory.

Wachter opened the meeting with a review of recent work by his team on semiconducting intermediate valence compounds. Because of the weak interactions of electrons in the f shells of adjacent rare-earth atoms, the characteristic energy gaps are of order 10 meV, corresponding to temperatures of order 100K. These are similar to energies associated with magnetic ordering and far-infrared optical absorption, so that small temperature changes can induce substantial changes in many physical properties. The metal-insulator transition under pressure is accompanied by a volume change of order 10 per cent, because of the different size of the rare-earth atom in its different charge states. As an incipient instability, it causes the elastic properties to be anomalous under ambient conditions. Compressibilities are enhanced, some elastic constants are negative and finite wavelength vibrational instabilities are encountered. G. Saunders (University of Bath) reported on these anomalies as the transition is approached. Possible applications as pressure sensors and phonon generators can be envisaged.

The rare-earth metals and their alloys (including those exemplified by $\text{Eu Cu}_2\text{Si}_2$) have optical, magnetic and electronic

transport properties that vary as the valence changes (reviewed by D. Wohlleben, University of Köln, and F. de Boer, University of Amsterdam). The valence changes generally take place at low temperatures, so that in ambient conditions most physical properties, though anomalous, are 'quiet'. The metallic alloys are easier to prepare, are simpler to analyse and are proving a fertile testing ground for the current theories of intermediate valence phenomena.

Preparation of pure and stoichiometric sulphides of rare-earth metals is proving difficult (H. Bach, University of Bochum), especially because of the high volatility of sulphur with respect to the rare-earth metals.

The low-temperature electrical conductivity of SmB_6 , and its sample dependence in particular, pose unanswered questions that impinge on the transport theories of disordered systems (Sir Nevill Mott, University of Cambridge). The magnitude of the resistivity of TmS and its relative temperature independence below 1K is a further puzzle. Attempts to extract an effective one-electron theory from the general many-electron formulation, in order to account for the anomalies in low-temperature specific heat and magnetic susceptibility of the metals, were reviewed by D. Newns (Imperial College). Mixed valence materials are those where one species is present in one of two charge states, and they have already found applications, particularly as dyes. P. Day (University of Oxford) cited many other examples, including Fe_3O_4 (magnetite) and LiTi_2O_4 (which goes superconducting).

Other points raised in the open-ended discussion included the inability of the compounds to survive more than ten cycles through the pressure-induced phase transition without damage, and the fact that doping (apart from alloying in metals) has not been investigated, as non-stoichiometry is already providing a wealth of phenomena. Catalytic properties have not been examined but were the subject of speculation.

Given our increased understanding of the semiconducting intermediate valence compounds, it remains for further advances in the methods of preparation to be achieved, and a wider characterization undertaken, before they replace existing materials in device applications or forge a new range of devices based on their unique combination of physical properties. □

*The meeting was held at the Long-Range Research Laboratory at the GEC Hirst Research Centre, East Lane, Wembley, UK, on 15 January 1982.

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REVIEW ARTICLE

Antibody diversity in lower vertebrates—why is it so restricted?

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Recent studies have revealed that lower vertebrates such as fish, amphibians and reptiles have an antibody repertoire that is much less diverse than that of mammals. It is suggested that the various mechanisms generating antibody diversity are the same in all vertebrates but that non-immunological parameters such as mode of ontogenetic development and cell cycle properties allow fewer somatic events in generating the antibody repertoire of cold-blooded vertebrates.

IMMUNOLOGISTS have always been puzzled by the extent of antibody diversity in mammals, in which the introduction of a single antigenic determinant can stimulate the immune system to produce hundreds of different antibodies. It is difficult to estimate the degree of the huge diversity apparent from these observations: on the one hand counting the genes that encode immunoglobulin gives numbers corresponding roughly to the potential diversity of the system but it is unknown whether these numbers correspond to as many products; on the other hand, by counting products, researchers cannot be sure they are not counting only predominant antibodies, and missing a large number of poorly expressed (therefore undetectable) antibodies. Estimates usually range from 10^7 to 10^9 or even more antibodies, speculation in higher vertebrates not being limited by the size of the immune system itself.

We now know that during the ontogenetic development of mouse lymphocytes, various DNA segments assemble and generate a great diversity of genes encoding the variable part of the heavy (H) or light (L) chain of the immunoglobulin molecule. The diversity of the antigen-binding sites resulting from these somatic DNA rearrangements depends on the number of sets and on the number of genes per set. In the most complex case, that of the heavy-chain variable region, the sets V (variable), D (diversity) and J (joining) are assembled to C (the constant-region segment) to form a complete VDJC immunoglobulin heavy-chain gene. There are probably a few hundred V germ-line genes, five J genes and an unknown number of D genes. Since any V gene can assemble apparently with any of the D segments and subsequently to any of the J segments, the diversity generated from this combination is great. It is further amplified by random assembly of the different V heavy chains with light chains to form the complete antigen-binding site. If, in addition, there appear somatic variants due to somatic mutations in the V genes or to joining 'mistakes' during the somatic rearrangement, the potential diversity is further increased (for review see ref. 1). This mechanism, which in mammals uses a large number of inherited germ-line genes and somatic diversification, brings together the once conflicting theories formulated to explain the origin of antibody diversity. However, recent investigations in lower vertebrates indicate that although the antibody repertoire of fish, amphibians, reptiles and perhaps even birds is heterogeneous, it is far less so than in mammals. Two questions immediately arise: (1) does this mean that diversification is achieved by different means in lower vertebrates and in mammals? (2) How can a species survive with such a reduced antibody diversity, or is mammalian diversity necessary? Here I present briefly evidence for reduced heterogeneity of the antibody repertoire in lower species compared with mammals and suggest that the differences are due

not only to an evolution of the mechanisms of diversification but also to some non-immunological parameters, such as properties of the cell cycle and the mode of embryonic and larval development.

Low antibody diversity of fishes

Antibody diversity of lower vertebrates has been studied so far by indirect methods based on structural studies, affinity measurements during the immune response, counting isoelectrofocusing (IEF) spectrotypes and by idiotype analysis. No estimate of gene numbers has yet been made in lower vertebrates where immunoglobulin gene analysis is still in its infancy. Some of the methods used are self explanatory: the greater the structural diversity, the number of IEF spectrotypes or the number of idiotypes, the greater the antibody diversity. Affinity measurements have been used to detect an increase in affinity (or maturation of the antibody response). Maturation occurs in mammals and has been interpreted as the consequence of the successive activation of B-cell clones producing different antibodies of increasing affinity. The occurrence of maturation thus implies substantial antibody heterogeneity.

In mammals, a second manifestation of immune response maturation is the successive expression of different isotypes (that is, classes) of immunoglobulin during the immune response. This does not occur in primitive cartilaginous fish, such as the shark *Heterodontus francisci*, which makes antibodies of the (immunoglobulin M) IgM class only. Both biological and structural data indicate that the repertoire of this species is low. Immunized with 2-furyloxazolone-*Brucella*, they mount a low-affinity antibody response which varies very little between the different individuals tested and does not increase in affinity after immunization². Although the modified bacteriophage inactivation assay used in these experiments is not ideal for heterogeneity studies because it detects only the predominant antibody in the serum, these results suggest a low antibody heterogeneity both in the species and in individuals; furthermore, the response of this species to a different antigen, *p*-azobenzenearsonate³, has similar characteristics. Limited sequence data on this horned shark immunoglobulin have shown that the variable region appears to be organized in the same way as in higher vertebrates, that is, with a framework and hypervariable regions^{4,5}. The variation between the light chains isolated from different individual animals is, however, very limited, with the major bands having identical isoelectric points. In slight contrast to these results, another species of shark, *Ginglymostoma cirratum*, immunized with heat-killed pepsinized streptococcal A variant vaccine, produces antibodies that among six outbred individuals have very different light chain gel electrophoresis characteristics⁶. However, as in

Heterodontus, no increase in variation occurs with time after immunization. Interestingly, in *Ginglymostoma* the heterogeneity of ligand binding involves intramolecular heterogeneity at the conformational level⁷, meaning that a single molecule could have dual specificities.

Although heterogeneous (that is, more than one antibody per antigen), the anti-dinitrophenol (DNP) response of a bony fish, the carp, consists of IgM antibodies that are all distributed between pH 4 and 6.4 on IEF. The number of IEF spectrotypes (1 spectrotypes = 1 antigen-binding band) per individual is small (up to 23) and there is little variation from one outbred individual to the other⁸. There are always several bands observed per antibody type on IEF gels because of deamidation, and in the absence of a reference fish monoclonal immunoglobulin, it is impossible to say how many antibody types the IEF bands correspond to, but certainly fewer than 23. Moreover, some anti-DNP cross-reactive idiotypes are present at a high frequency in an outbred carp population (in all of 11 immunized animals whereas all preimmune sera were negative)⁹. This does not necessarily mean that the responses of the bony fish lack specificity: their capacity to recognize two Waldenström protein mammalian idiotypes is similar to that of guinea pigs^{10,11}. If mammalian idotype diversity were equivalent to immunoglobulin diversity, such a result could mean that the fish repertoire is approximately as large as that of the mammal. However, many mammalian immunoglobulins have cross-reactive or even identical idiotypes and differ elsewhere in the molecule, so that idotype diversity may be much smaller than immunoglobulin diversity. Moreover, it is unknown whether the fish anti-idotype antibodies are as heterogeneous as those of the guinea pig.

Amphibian immunoglobulin heterogeneity

In amphibians the primitive urodeles express a very restricted repertoire of antibodies, all of the IgM class as in fish¹². They do not respond well to thymus-dependent antigens¹³, which may be due to a lack of T-cell help but could also be due to the limited repertoire. The fact that there are high levels of natural antibodies to several antigens supports the latter hypothesis: if there are few categories of antibodies, all of them are represented at a high concentration in the serum. More data are available for anurans, particularly *Rana* and *Xenopus*. Early work¹⁴⁻¹⁷ on tadpoles proved that anuran larvae could respond specifically (with a number of lymphocytes of the order of 10^6 cells) to many antigens such as bacteriophages, mammalian red cells, protein antigens and hapten-carrier immunogens like DNP and keyhole limpet haemocyanin (KLH); the maturation of the IgM anti-DNP response was also demonstrated in tadpoles and in adults^{18,19}. Larval and adult frogs responded in a heterogeneous manner, as proved by IEF analysis^{17,20,21}. However, the heterogeneity of the response to red cells, DNP or phosphorylcholine is much more restricted than in mammals. The number of IEF spectrotypes per individual varies from 4 (phosphorylcholine) to 20 (anti-DNP) and very probably corresponds to fewer antibody categories. In the species the number of different anti-DNP antibodies may not exceed 40. These numbers are low compared with those recorded in mammals where, for example, anti-DNP spectrotypes can number as many as 500 (refs 22, 23).

In *Xenopus*, as in the shark, the amino acid sequence indicates low antibody heterogeneity^{24,25}: heterogeneous anti-DNP or even non-immune immunoglobulin pools have provided easily interpretable sequences for the first 16 N-terminal residues of both heavy and light chain variable regions. Altogether, the antibody diversity of anuran amphibians has been estimated at 5×10^4 – 5×10^5 different antibody molecules.

The availability of clones of *Xenopus* has allowed examination of the role of genetically inherited material in the expression of antibody diversity. Isogenic *Xenopus* produce antibodies to DNP, xenogeneic red cells, or phosphorylcholine whose IEF spectrotypes are identical or very similar between

isogenic individuals, whereas they may differ among outbred individuals. The idiotypes of the anti-DNP antibodies cross-react to such an extent that they have been considered identical. Both IEF spectrotypes and idiotypes are inheritable through several generations of cloned animals, suggesting that diversity is not only reduced compared with mammals, but is due essentially to the expression of germ-line genes^{21,25}.

Reptiles and birds

Investigation of antibody diversity has been less extensive in reptiles and birds. In the former, the lack of or difficulty in detecting an increase in affinity suggests low heterogeneity²⁶. Isoelectric focusing data have suggested a low diversity of antibodies against human serum albumin and DNP in chicken²⁷. Sequence data may also be interpreted in support of this as chicken light chains seem, on two-dimensional gel electrophoresis, to be less heterogeneous than mouse light chains²⁸⁻³⁰. The poor increase in affinity of chicken antibodies to two haptens (DNP and fluorescein) may again indicate a lower heterogeneity of these antibodies³¹. However, the antibody repertoire of the chicken to streptococcal A vaccine is as large as that of mice³². In summary, it is difficult to compare frog and bird repertoires, because the only antigen for which heterogeneity has been studied in detail in birds and mammals (the streptococcal A vaccine) has not been analysed in frogs. The possibility remains that birds have an antibody diversity lower than that of mammals.

Discussion

To account for what seems a conspicuous difference between mammalian and lower vertebrate antibody repertoire, I shall first consider the known mechanisms for antibody diversification: (1) increase in diversity of a set of germ-line V, D, J genes by accumulation of germ-line mutations during phylogeny; (2) a mechanism of diversification during ontogeny by combining the various V, D and J elements at random; and (3) a mechanism of diversification by somatic mutation, also during ontogeny.

One may first consider the possibility that in lower vertebrates the immunoglobulin genetic system is simpler than in mammals, for example, that the J or D segments are missing or rather that their homologues are not yet independently duplicated but have remained associated with some part of the variable immunoglobulin gene. These animals may also have fewer genes per category, that is, fewer V, J and D segments.

I suggest that the mechanisms of somatic diversification which operate during ontogeny also occur in lower vertebrates, but that they are influenced by elements outside the immune system, such as cell cycle properties and mode of embryonic development, and that these influences may reduce the effect of one or other somatic diversification mechanism, thereby giving the impression that most antibody diversity is of germ-line origin. Given the great homology of structure between primitive fish IgM and mammalian IgM³³ and the homology between the genes for *Xenopus* and mammalian IgM³⁴, it is likely that immunoglobulin genes in lower vertebrates are organized in a way similar to that of mammals, that is, with a rearrangement of gene segments during lymphocyte ontogeny.

The expression of antibody repertoire can then be influenced by the following. (1) How much wastage of lymphocytes is possible in animals in which lymphocytes proliferate quickly, cell numbers are large, and nonsense mutations can occur without jeopardizing survival; then somatic mechanisms for increasing diversity and especially somatic mutation may be not only tolerated but favoured. This is the case in mammals where lymphocytes can be considered 'cheap'. If cells do not proliferate quickly, if the number of lymphocyte generations is small compared with the life of the individual, or if lymphocyte numbers are small, then the somatic events, even if they occur at the same frequency as in mammals, will have a less important

role. The species will then *de facto* rely more on its set of germ-line genes and any mutants that may occur among them. This situation occurs in cold-blooded vertebrates, where the cell cycle can be prolonged when temperatures are low³⁵, where cells are often big and hence in smaller numbers, and thus where lymphocytes cannot be wasted and can be considered 'expensive'.

(2) In such species there is even more pressure for the quick expression of a repertoire of germ-line genes because development generally occurs outside the mother and remarkably quickly. If an efficient immune system is not developed within a few days after fertilization, the individuals may be easily infected by external pathogens. Then their immune system has no time to select randomly appearing somatic mutants of lymphocyte clones. It seems likely that their protection will be best ensured by the rapid expression of a selected set of germ-line V genes.

Finally, how can lower vertebrates accommodate a lower antibody diversity and is mammalian diversity a necessity? One may consider that the immune system of lower vertebrates has been submitted to less pressure for diversification because of the existence of non-immunological defence mechanisms which they inherited from invertebrates. There exist in

invertebrates^{36,37} and lower vertebrates³⁸ such mechanisms as natural haemolysin and complement-related compounds which can recognize cell surfaces of bacteria or xenogeneic cells, lyse these cells and hence may prevent the immune system of the animals from seeing many antigens, thereby reducing the external pressure for diversification of antibody repertoire.

Although considerably smaller than those of mammals, the antibody repertoires of lower vertebrates can still be considered very efficient as most antigens can be recognized by a lower vertebrate immune system having a repertoire of possibly at most 5×10^5 antibody varieties. This raises the question of the need for the great diversity of mammalian antibodies. It is not proved that all antibody varieties are necessary, some may be unselected products which could be inherent in any system in which there are somatic rearrangements.

It is also possible that evolution of diversification may move back and forth and there is no indication that the state of antibody diversification known in mammals is the best and final form. We may be observing them in a phase of expansion of antibody diversity preceding a phase of contraction.

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ARTICLES

Evolution of the Iceland hotspot

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Mantle geochemical enrichment beneath the Iceland–Faeroes hotspot has been episodic, suggesting that blobs rather than a continuous plume may be rising in the region. Recurring rift propagation southward from central Iceland causing progressive melting of the lower crust followed by the upper mantle, best explains the geochemical variations found along the south-west and south-east neovolcanic zones and their degree of thermal maturity.

ALTHOUGH there is no agreement on a particular model for the origin of Iceland, astride the Mid-Atlantic Ridge, it is generally accepted that Iceland represents an anomalously elevated segment of the Mid-Atlantic Ridge where unusual processes and conditions are superimposed on those associated with simple seafloor spreading.

For example, the Iceland crust is thicker and its structure and composition distinct from that of typical oceanic crust^{1–4}. The melting zone beneath Iceland seems to extend to greater depth, volcanism is more intense, and its products greatly more

varied than along the submerged part of the Mid-Atlantic Ridge².

The composition of the mantle beneath Iceland is apparently also richer in volatiles and radiogenic isotopes of Sr and Pb than the surrounding depleted asthenosphere (that is, the source of normal mid-ocean ridge basalts)^{2–7}. Some compositional zoning in the mantle about Iceland may also be present^{2–4,8}.

To complicate matters, geological processes by which Iceland developed do not appear to have been constant in intensity nor necessarily continuous in time. For example, palaeomagnetism

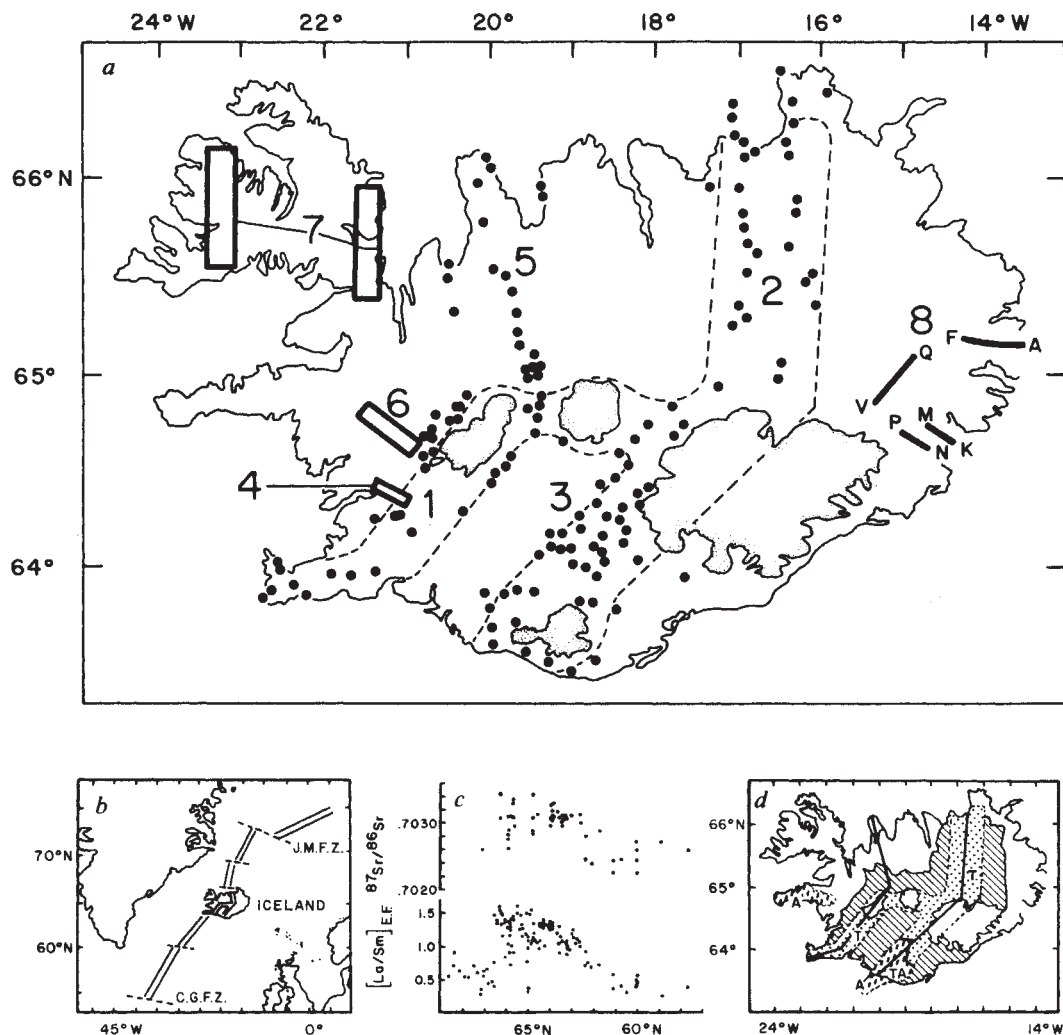


Fig. 1 a, Iceland map, showing location of samples in volcanic zones and regions studied: 1, south-west zone (53 samples); 2, north-east zone (37 samples); 3, south-east zone (59 samples); 4, West Iceland (33 samples); 5, Skagi^{18,19}; 6, Borgarfjörður (29 samples); 7, Vestfirðir (68 samples); 8, eastern Iceland (41 samples; letter profiles are from ref. 28). b, Iceland and its relation to adjacent Mid-Atlantic Ridge segments. c, Variation of $^{87}\text{Sr}/^{86}\text{Sr}$ and (La/Sm) along the Kolbeinsey Ridge, the north-east and south-west zones and the Reykjanes Ridge. Sr isotope data are from refs 3, 17, 23. d, The distribution of petrological types after refs 35, 36. The jagged line across the south-east transitional alkali basalts with $(\text{La}/\text{Sm}) > 1.75$ from tholeiites with $(\text{La}/\text{Sm}) < 1.75$. Central axes through the volcanic zones used for data projection in Fig. 5 are based on the orientation of fissure swarms and volcanic systems^{32,36}.

stratigraphical studies⁹⁻¹¹ and detailed mapping suggest that the volcanic eruption rate has been variable. In addition, the tectonic evolution of Iceland has been complicated by complex rifting zones jumping occurring intermittently throughout its history, sometimes even producing two parallel rifting zones with activities overlapping in time such as the present south-east and south-west neovolcanic zones (Fig. 1)^{1,12-16}. The presence of the East Greenland-Iceland-Faroes Ridge, however, suggests that such unusual activities have been long-lived, and possibly episodic. O'Nions and Pankhurst¹⁷ have also observed a secular decrease in $^{87}\text{Sr}/^{86}\text{Sr}$ in Iceland rocks of age ranging from 16 Myr BP to the present, suggesting that the source of Iceland volcanic products may have varied with time.

Here we review the geochemical data we have accumulated in the Iceland-Faroes-East Greenland region, emphasizing spatial and temporal rare earth (RE) variations in basalts, as well as comparing known Icelandic rift zones at apparently different thermal and tectonic stages of development. For example, the north-east and south-west zones, which are current landward extensions of the Mid-Atlantic Ridge axis (Fig. 1), are considered to be mature rift zones at a quasi-steady state, exhibiting high heat flow and well-developed extensional tectonics¹. On the other hand, the south-east zone, a flank zone parallel to the south-west rift zone, appears to be a younger rifting zone in the process of propagating southward. This rift zone does not exhibit any heat flow anomaly and has only poorly developed extensional features¹. It seems to be at a transient stage of development. In contrast, the Skagi zone appears to be an ephemeral palaeorift which developed ~2.5 Myr BP on the flank of the main rift zone in North Iceland, and subsequently died without reaching maturity^{18,19}. In con-

trast to the south-east zone, there is no petrological and geochemical evidence suggesting that this ephemeral rift developed by propagation but rather evolved uniformly along its entire length.

We will demonstrate that volcanic products associated with rift zones at such distinct stages of development are also petrologically and geochemically distinct. If this interpretation is correct, we can begin to decipher the thermal and tectonic evolution of Iceland in the more remote past.

Our report is based on rare-earth concentrations (La, Sm and Yb) of 334 basalts broadly distributed throughout Iceland (Fig. 1), and isotopic data published^{3,4,17,20-27}. (La/Sm) and (Yb) indicate chondrite normalized enrichment factors for La/Sm and Yb. A good age control for Pleistocene-Miocene lavas was obtained in eastern Iceland, Vestfirðir, Borgarfjörður and West Iceland by coordinating our sampling with existing age dating and palaeomagnetostatigraphic studies of these regions^{9-11,28-30}. Part of the RE data has been published elsewhere^{2,19}, and the remaining RE analyses can be found in ref. 31.

Temporal variations

Rare-earth patterns of all the Iceland basalts studied are shown in Fig. 2a. There is an important difference in the type and range of pattern for Pleistocene-Miocene (13.5-0.7 Myr) basalts and basalts from the neovolcanic zones (<0.7 Myr). Pleistocene-Miocene lavas are consistently enriched in light-RE, whereas more recent lavas exhibit a wider spread in light-RE from enriched to depleted patterns more like those of normal mid-ocean ridges. The (La/Sm) distribution in Pleistocene-Miocene lavas is normal with a mode around 1.65

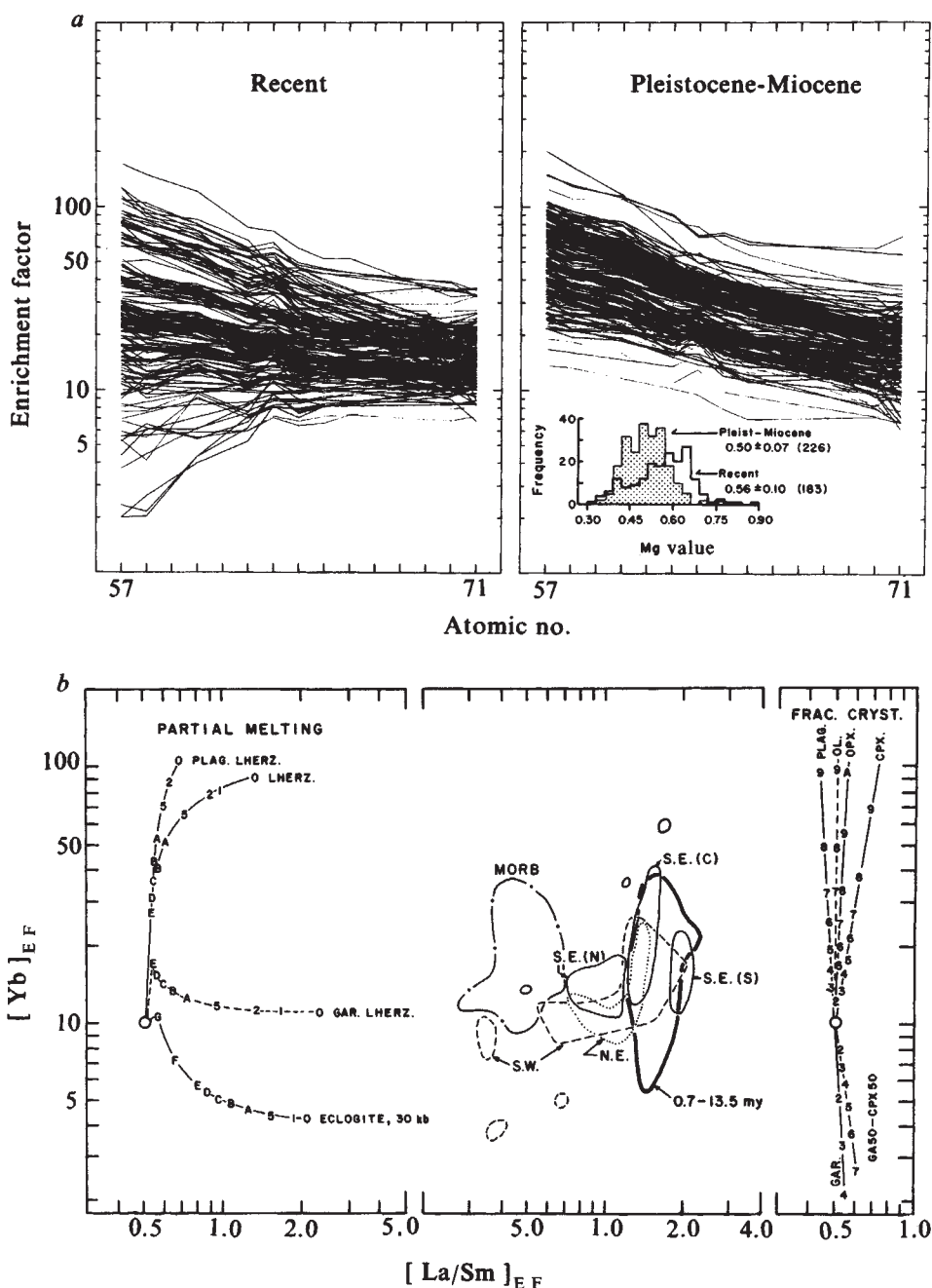
and an average of 1.7 ± 0.27 (1 s.d.), whereas the (La/Sm) distribution in Recent lavas tends to be positively skewed with a mode around 1.35 and an average of 1.34 ± 0.40 . There is also a significant statistical difference at the 99% confidence level in the distribution of Mg-value of Pleistocene-Miocene lavas which is normal with a mode around 0.5, and that of Recent basalts which has a mode around 0.6 and is positively skewed (Fig. 2a). Figure 3 shows that light RE-depleted basalts $\{(La/Sm) < 1\}$ occurring in Recent time are confined to basalts high in Mg-value ($Mg/Mg + Fe^{2+}$, atomic concentration ratio) representing either olivine tholeiites or picritic basalts as also found by other authors^{18,19,25}. Finally, the new Miocene picritic basalts we have encountered in Vestfirðir are light-RE enriched and not depleted as in Recent time. Thus, light-RE depleted basalts seem to reflect eruption of a basalt type which has clearly developed on Iceland only in Recent time.

The above results suggest a secular decrease in light-RE enrichment from Tertiary to Present which is best illustrated in the Eastern Plateau basalt succession (Fig. 4). A broad regular decrease is readily noted from 13.5 to 2 Myr. Extrapolation of this trend below 2 Myr BP to the zero age intercept coincides

with the mean value found for the north-east neovolcanic zone, which is the locus of the present spreading axis in northern Iceland. The secular variation extends even within the north-east zone where the dormant east fissure swarm is dominated by light-RE enriched basalts, whereas light-RE depleted patterns are more predominant in the now active western fissure swarm mapped by Walker³². Thus this is also apparent in West Iceland between basalts greater and < 0.7 Myr BP. The rate of light-RE decrease seems to have increased significantly within the past 0.7 Myr BP.

In addition to the secular decrease in (La/Sm), there is a concomitant secular decrease in $^{87}Sr/^{86}Sr$ throughout Iceland suggesting that the mantle source of these basalts beneath Iceland has evolved¹⁷. Such secular geochemical variations are not unique to the past 14 Myr history of the Faeroes-Iceland-Greenland Ridge, but has also been observed during the later period of the Faeroes Island Plateau basalt formation some 55–50 Myr BP, at the onset of continental drift in this region of the North Atlantic^{26,33}. (La/Sm) and $^{87}Sr/^{86}Sr$ decrease and $^{143}Nd/^{144}Nd$ increases as one proceeds from the lower and middle basalt series of the Faeroes^{26,31}. The Plateau basalts

Fig. 2 a, Rare-earth patterns for Recent basalts (< 0.7 Myr BP) and Pleistocene to Miocene basalts (0.7–13.5 Myr BP). Enrichment factor refers to REE concentration relative to chondrites. Histograms compare the distribution of Mg-values corresponding to these two populations. b, (Yb) against (La/Sm) variation for Iceland basalts compared with theoretically derived RE variations generated by different degrees of partial melting (batch melting) of various mantle sources (left) and different degrees of fractional crystallization (right). The field for Pleistocene-Miocene basalts (solid line labelled 0.7–13.5 Myr) is based on data from the Eastern Iceland Plateau, Vestfirðir, Borgarfjörður and West Iceland basalts > 0.7 Myr old. SE (N), SE (C) and SE (S), northern, central and southern part of the south-east zone, respectively. SW, south-west zone; NE, north-east zone. MORB, normal mid-ocean ridge basalts from the Kolbeinsey and Reykjanes Ridges. The starting composition for partial melting and fractional crystallization models was arbitrarily set at (Yb) = 10 and (La/Sm) = 0.5. Plag., lherz., lherz., gar. lherz., and eclogite plagioclase lherzolite source (20% plagioclase, 48% olivine, 16% orthopyroxene, 16% clinopyroxene), a lherzolite source (60% Ol, 20% Opx, 20% Cpx), a garnet lherzolite source (48% Ol, 16% Opx, 16% Cpx, 20% Gar) and an eclogite source (50% Cpx, 50% Gar). 0, 2, 5 and A, B, C, D, E, F, G along the partial melting curves indicate 0, 2, 5, 10, 15, 20, 25, 30, 50 and 77% melting. 2, 3, 4, 5, 6, 7, 8, 9, A along the fractional crystallization curves indicate 20, 30, 40, 50, 60, 70, 80, 90 and 95% crystallization of plagioclase (Plag), olivine (Ol), orthopyroxene (Opx), clinopyroxene (Cpx), garnet (Gar) and an eclogitic mixture (Ga 50–Cpx 50). Partition coefficients used are as given in ref. 71.



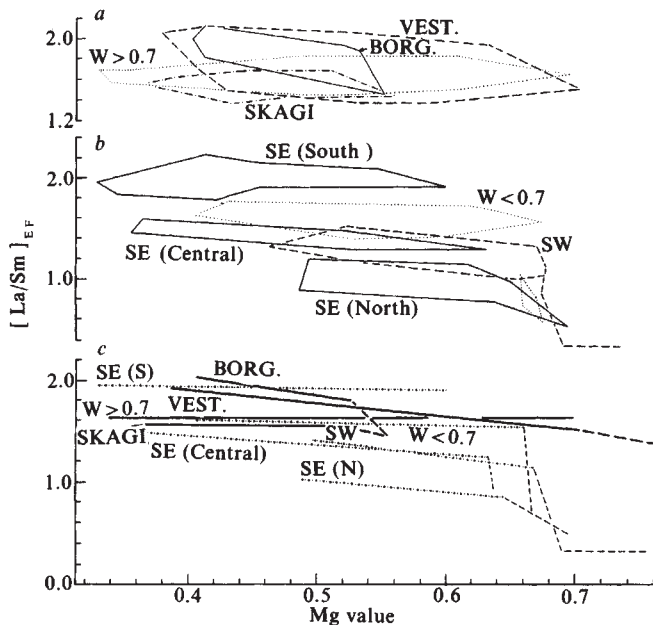


Fig. 3 Co-variation between Mg-value and (La/Sm) for individual zones. *a*, Variations in the Pleistocene-Miocene zones; Vestfirðir (VEST.), Borgarfjörður (BORG.), Skagi and West Iceland (W) >0.7 Myr. *b*, Variations in the Recent zones; south-west (SW), south-east (SE) and West Iceland (W), 0.7 Myr. *c*, Variations in the Recent zones; south-west (SW), south-east (SE) and West Iceland (W), 0.7 Myr. *c*, Miocene zones (bold solid lines).

from East Greenland's continental border are similar to the Faeroes upper series, suggesting symmetry about the Mid-Atlantic spreading axis³³. We conclude that the secular (La/Sm) observed both in eastern Iceland, and earlier in the Faeroes, reflect a true secular variation in the composition of the mantle source of these basalts. The enrichments seem to have been episodic during the evolution of the Iceland hotspot evolution at least over the past 60 Myr.

The secular variations shown in eastern Iceland may not be totally devoid of complexity due to spatial variations (Fig. 4). A close look at the broad (La/Sm) secular decrease, may indicate a variation of a shorter wavelength, with a maximum around the 7–8 Myr period. Such a perturbation may be significant as it coincides with a period of high lava extrusion rate^{9,11} and the proposed sudden increase in volcanic activity on the Reykjanes Ridge which apparently initiated the development of time transgressive V-shaped ridges³⁴. However, there is also a 60-km break in the sample profile at this point, and thus the perturbation could also reflect some small but significant spatial variations existing at that time.

Figure 4 further shows that the regular secular trend noted in the Eastern Iceland Plateau is generally not corroborated in western Iceland based on composite basalt profiles from widely separated regions, though basalts from Vestfirðir are statistically indistinguishable from the Eastern Plateau basalts over the same 13.5–9 Myr BP period. This may partly be due to our inadequate sampling which does not permit us to take into consideration possible Tertiary spatial variations along rift strikes, such as those currently found along the neovolcanic zones, and partly due to complications imposed by rift jumping and the probable presence of parallel ephemeral rift zones such as Skagi and possibly Borgarfjörður.

Spatial variations

For practical reasons, our study of spatial variations across Iceland has been limited to known palaeo- or neovolcanic rift zones. We have projected the variation in (La/Sm) and Mg-value within these zones along two major profiles running

essentially parallel across Iceland (Fig. 1): the Eastern and Western Profiles (Fig. 5). Note that all the lavas from the Eastern Profile are essentially contemporaneous, whereas there is an age discontinuity on the Western Profile corresponding to Skagi (0.5–2.5 Myr) and the south-west zone (<0.7 Myr). Clearly there are important RE and Mg-value variations between the neovolcanic zones as well as within them. (La/Sm) and Mg-value respectively decrease and increase northward along both the south-east and the south-west zone, but at a faster rate in the SW zone. On the other hand, ⁸⁷Sr/⁸⁶Sr appears relatively constant along the entire south-west zone³, but tends to increase southward along the south-east zone^{3,20,21,23,27}. The increase in (La/Sm) along the south-east zone is rather stepwise, and the variation essentially reflects three major petrological zones recognized first by Jakobsson (Fig. 1)^{35,36}. This is not the case along the south-west zone which shows no petrological gradient and is dominated by olivine tholeiites and tholeiites³⁷. In contrast, the (La/Sm) and Mg-value variations in the Skagi palaeorift zone and north-east neovolcanic zone show no well developed spatial trends along strike^{18,19}, although there is a lateral difference in the distribution of RE patterns between the dormant western and the active eastern fissure swarms of the north-east zone³².

Finally, our extensive survey demonstrates that the strongly light-RE depleted picritic basalts previously reported in Central Iceland^{20,23,27} and the Reykjanes Peninsula³⁷ are, in fact, limited to these two regions which have been proposed to be fracture zones^{1,18} (Fig. 5). None were found elsewhere along the neovolcanic zones nor in the Pleistocene-Miocene lavas of Iceland. Only three picritic basalts were observed in our survey of Pleistocene-Miocene lavas, but these have light-RE enriched patterns. The spatial contrast within and between known rift zones of Iceland is further illustrated in (La/Sm) versus Mg-value plots (Fig. 3). The (La/Sm) appears to be insensitive to

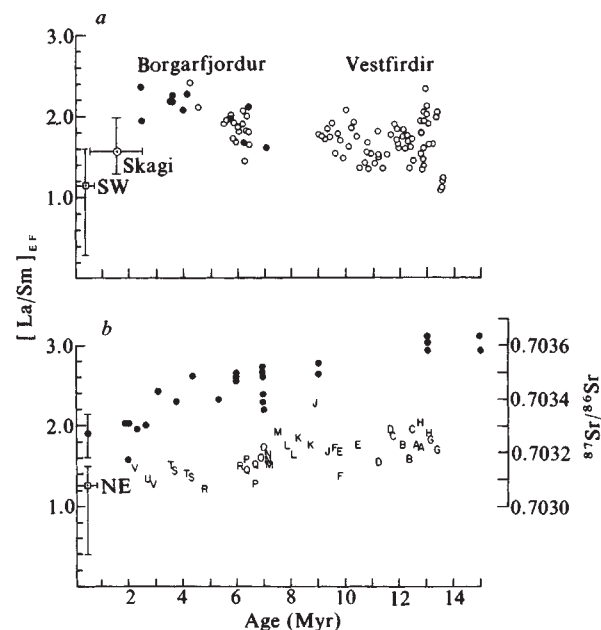


Fig. 4 Secular variation of (La/Sm) through the basalt plateaus in western (*a*) and eastern (*b*) Iceland. In *a*, ●, samples dated by the K–Ar method¹⁰; ○, samples whose age has been estimated from their stratigraphical height and the assumed linear relationship between stratigraphical height and age from ref. 10 for Borgarfjörður and McDougall and Kristjánsson (in preparation) for Vestfirðir. Ages between 13.5 and 10.5 Myr are based on the average K–Ar ages of several profiles while ages between 10.5 and 9.0 Myr are based entirely on field relations relative to older, dated lavas. The average (La/Sm) for the south-west (SW), and Skagi zones are shown for comparison, error bars corresponding to the range in age and (La/Sm) observed in each zone. In the eastern Iceland series, letters refer to different profiles (see Fig. 1). Ages of samples in this series are based on the palaeomagnetic stratigraphy in ref. 11. The average (La/Sm) in the north-east (NE) zone is also shown. ⁸⁷Sr/⁸⁶Sr data (●) is from ref. 17.

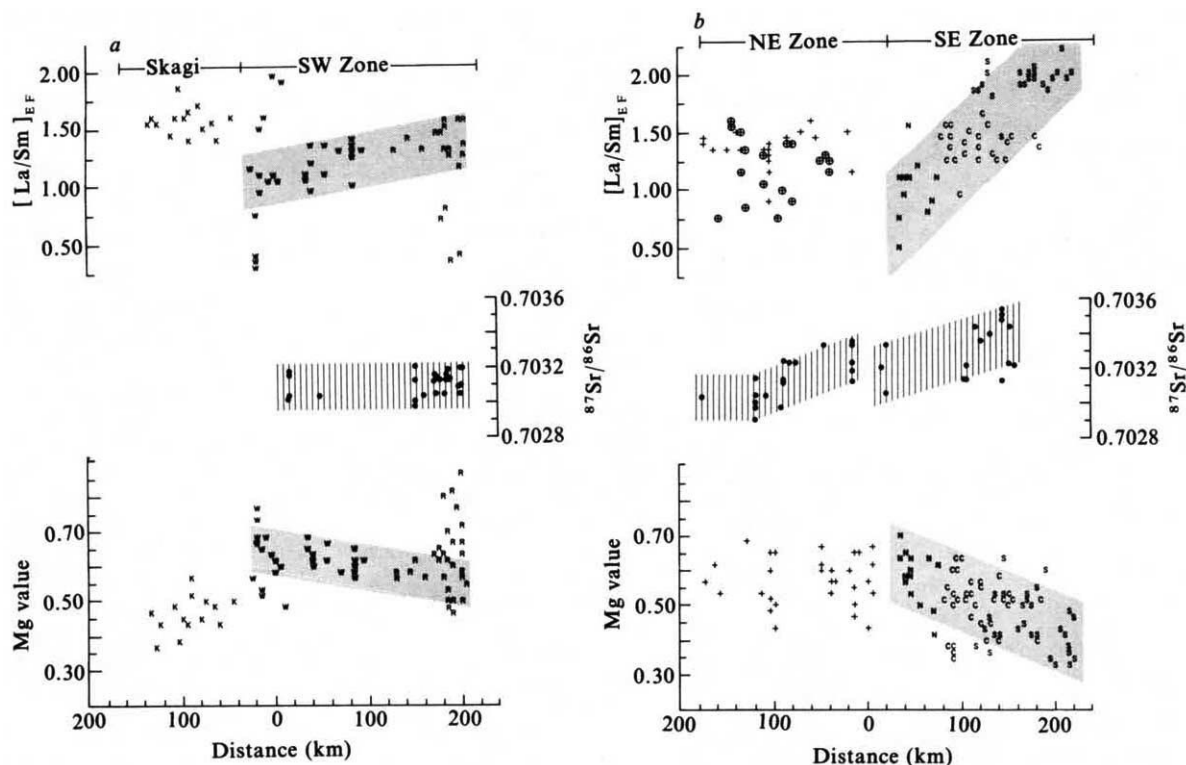


Fig. 5 (La/Sm), Mg-value and $^{87}\text{Sr}/^{86}\text{Sr}$ variations along: *a*, western (SW and Skagi); *b*, eastern (SE and NE) volcanic zones. Distance along the abscissa refers to distance away from central Iceland 64.86°N along the axes of the volcanic zones (see inset Fig. 1). Sample locations have been projected normally to zone axes. Samples from the active western $\bullet$ and dormant eastern $+$ part of the north-east zone are indicated. Mg-value data for the north-east zone is from ref. 72, whereas the $^{87}\text{Sr}/^{86}\text{Sr}$ variations were constructed from data in refs 3, 4, 17, 20–25 and 27.

Mg-value variation in a particular zone, but the level of (La/Sm) is distinct from one zone to another.

We consider that the distinct (La/Sm) level within each region reflects similar mantle source variations. On the other hand, the different (La/Sm) levels observed within the south-east zone, which also corresponds to three different petrological types^{35,36}, suggest both distinct sources as well as melting conditions.

Model constraints

Although numerous models have been proposed to explain trace element and isotopic variations in isolated regions in Iceland (see refs 22, 23, 25, 38–40), few have been suggested for the entire Iceland plateau and surrounding ridge segments that account for both spatial and temporal variations in a unified way.

In general, processes which have been suggested to affect RE patterns in Iceland basalts range from: (1) fractional crystallization; (2) partial melting conditions in the mantle (such as degrees of melting, mineralogy and dynamics of melt removal); (3) magma–wall rock interaction; (4) zeolitization and spatial variations within eruptive lenses^{41,42}; (5) mixing of different magmas or reservoirs; (6) mantle heterogeneities (of specified or unspecified causes).

Merits of these possibilities are briefly evaluated before we propose our preferred model.

(1) Although it is clear that fractional crystallization took place from the presence of phenocrysts, large (Yb) variation and Mg-value variation within individual zones (Fig. 2*b* and Fig. 5), such a process cannot account for the (La/Sm) variation observed within a particular region. This is also ruled out in any region where there is a covariation of (La/Sm) and $^{87}\text{Sr}/^{86}\text{Sr}$, assuming that equilibrium rather than disequilibrium partial melting takes place (Figs 4 and 5).

(2) Variation in degree of batch partial melting is also unlikely to account for the secular variation in RE patterns noted in Figs 2*a* and 4 as the range of partial melting would be from a few per cent to 100%, and thus is untenable petrologically speaking, considering that the mantle is most probably peridotitic. Of course, some variation in partial melting is likely to take place and is permissible depending on the existing thermal regime, type of lava erupted, and isotopic ratios observed²⁷. The south-west zone is an example where (La/Sm) decreases towards Central Iceland, whereas $^{87}\text{Sr}/^{86}\text{Sr}$ remains essentially constant (Fig. 5), and the south-east zone is an example where $^{87}\text{Sr}/^{86}\text{Sr}$ and (La/Sm) co-vary and the petrological basalt type changes from tholeiitic to alkalic (Fig. 5).

Wood's model⁴³ invoking fractional melting is also unlikely to explain the secular variation observed for both the RE and $^{87}\text{Sr}/^{86}\text{Sr}$ (or $^{14}\text{Nd}/^{144}\text{Nd}$). It is sufficient to say that the build-up of $^{87}\text{Sr}/^{86}\text{Sr}$ with time is irreversible. Once a source region high in $^{87}\text{Sr}/^{86}\text{Sr}$ has been established, successive partial melting of such a source region in equilibrium conditions will not decrease this ratio (which has been corrected for any possible isotopic mass fractionation, see ref. 3). It is also improbable that models which consider disequilibrium melting of a veined mantle^{27,44} can explain the observed secular trend over approximately a 10-Myr period of seafloor spreading and the cyclic nature of the Iceland hotspot during the past 60 Myr. This would require a mechanical decoupling of the crust generated by spreading and the mantle source from which melts are derived (that is, decoupling of the crust from the lower part of the lithosphere). This is totally inconsistent with plate tectonic theory, structure of the lithosphere and the distribution of earthquakes. Furthermore, application of the concept of such disequilibrium melting⁴³ for generation of basalts, which seem to have required rather large degrees of melting, does not seem viable on the basis of Hofmann and Hart's⁴⁵ kinetic and scaling analysis.

(3) It is difficult to imagine how magma–wall rock interaction could account for the regular spatial and temporal variation

and cyclic nature of the geochemical variation noted. Indeed, because very *ad hoc* conditions would have to be invoked we reject this possibility.

(4) Wood and co-workers⁴⁰⁻⁴² interpreted the RE variations observed in eastern Iceland in terms of spatial variations within eruptive lenses rather than temporal effects. Wood⁴¹ also suggested that the high (La/Sm) found in the oldest basalts was due to increasing depth or burial and zeolitization of the lava pile. We find Wood's explanations unlikely because: first, basalts of the same age from the Vestfirðir Plateau (9–13.5 Myr), which we have studied because they have not been zeolitized, also have high (La/Sm), indistinguishable from that found in basalts of the same age from eastern Iceland (Fig. 4). Second, Miocene picritic basalts from Vestfirðir are light-RE enriched and not depleted, as Wood suggests for high MgO basalts from shield volcanoes erupted near lens margins, which would have subsequently been eroded⁴². Third, the secular variation shown in Fig. 4 using the new dating scale of Watkins and Walker¹¹ is much smoother than that observed using the scale of Dagley *et al.*²⁸. This supports our view that the variation is indeed time-related, rather than spatial^{41,42}. Fourth, contrary to Wood's model predicting increasing (La/Sm) with increasing depth of burial and grade of zeolitization: our samples from both the N section within the highest grade laumontite facies and sections J to P in the lower grade mesolite facies fall on the general secular trend shown in Fig. 4, and not above; our basalts from sections A and B which are of similar age and geographical location have similar (La/Sm) despite the fact that section A has undergone a higher degree of metamorphism (mesolite facies) than section B (analcite facies) (Fig. 4); and

this is also the case for sections F and J. Finally, in addition to the secular decrease in (La/Sm), there is a concomitant decrease in ⁸⁷Sr/⁸⁶Sr and an increase in ¹⁴³Nd/¹⁴⁴Nd both on Iceland during (the past 13 Myr (refs 17, 24) and on the Faeroes 60–50 Myr BP (ref. 26). This is too much of a coincidence to be attributed to secondary alteration⁴¹, as for both Iceland and the Faeroes, the (La/Sm) and isotopic trends were established on totally different sample suites. Furthermore, the radiogenic Nd isotopic ratio is unaffected by weathering and mild metamorphism or metasomatism⁴⁶.

(5) Oskarsson *et al.*⁴⁷ have proposed a petrochemical model for rift zones on Iceland which is consistent with the crustal accretion model of Palmason⁴⁸. Basalt variations are explained by mixing between mantle derived olivine-tholeiites and variable amounts of crustal-derived magmas ranging from silicic to nepheline-normative melts. Flank zone volcanism^{35,36,38} is considered to be dominated by crustal-derived magmas, whereas the main axial rift zones are dominated by mantle-derived olivine-tholeiites (Fig. 1b). A crucial assumption of this model is that the subsiding crust becomes completely hydrated by hydrothermal activity. Studies of DSDP rocks of various ages in the oceans, show that hydration of the oceanic upper crust is very irregular and incomplete, despite the ample abundance of seawater above. It is doubtful that on Iceland where such an abundant source of fluids is absent, complete hydration would in fact take place. Their model also does not consider the temporal, nor spatial, variations found along the main axial zones discussed here. However, magmas of crustal derivation in the southern part of the south-east zone is an attractive

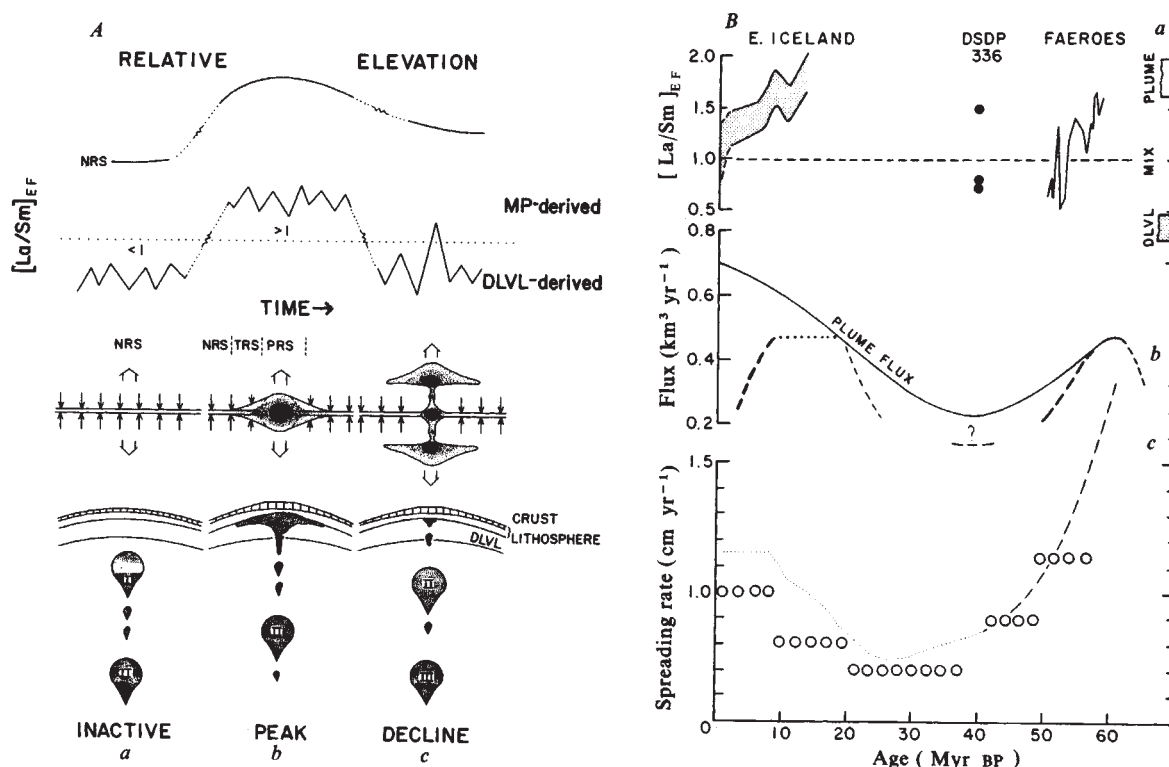


Fig. 6 A, model of a mantle plume (MP) ridge system showing variation in plume flux with time and a cycle in plume (blob) activity that is consistent with geochemical variations observed in erupted basalts. Phases: a, plume is rising beneath an existing spreading ridge (passive spreading); b, plume flux exceeds the gap created by spreading resulting in blob flattening and spill along spreading directions (active spreading). Plume-derived material dominates the erupted products which accumulate as plateau basalts; c, tailing phase of the blob. Plume flux is insufficient to feed the overlying spreading ridge and material from the depleted low velocity layer (DLVL) compensates. Small arrows indicate horizontal component of asthenospheric flow of DLVL material toward the ridge axis. Large arrows indicate spreading directions of the overlying lithospheric plates. NRS, TRS and PRS stand for normal, transitional and plume ridge segments. B, Reykjanes Ridge spreading rates, Faeroes-Iceland mantle plume flux and (La/Sm) variations with time modified from ref. 33. Curves c are spreading rates by Vogt and Avery⁵⁰ (dotted-dashed curve) and by Herron and Talwani⁵¹ (○). Curve b is the plume flux (solid line) estimated from Vogt and Avery, assuming basalts represent 25% partial melting and, therefore, that basaltic discharge is equal to one-quarter of the total plume flux. The dashed lines indicate our estimate of plume flux for the Iceland-Faeroe plume during the 60–50 (Schilling and Noe-Nygaard 1974) and 13.5–0 Myr periods, based on (La/Sm) as shown in A. ●, Data from DSDP site 336 (ref. 51).

hypothesis but the crust need not be hydrated to the extent indicated by these authors.

Preferred model

The binary mixing asthenosphere-plume or blob model proposed earlier (Fig. 6)³³ can readily explain the noted temporal and spatial co-variation of (La/Sm) and ⁸⁷Sr/⁸⁶Sr, but is inadequate when there is a decoupling between these two parameters, such as in the south-west zone (Fig. 5). It also fails to explain readily the southward (La/Sm) and ⁸⁷Sr/⁸⁶Sr decrease along the SE zone since it is of opposite sense to the gradient observed along the Reykjanes Ridge and Peninsula. We propose a composite dynamical model. The blob model explains the secular variation whereas spatial variation such as, for example, along the south-east zone is explained by rift propagation into older crust, resulting in, first, melting of the older large-ion-lithophile-enriched (LILE) lower crust-lithosphere, the enrichment reflecting an earlier stage in blob activity, followed by melting of the upper mantle due to advection.

In the blob model, the RE pattern of a basalt primarily reflects the mixing proportions between a light RE-enriched mantle plume source ($\{La/Sm\} > 1$) and the asthenosphere which is characteristically depleted in light RE ($\{La/Sm\} \leq 0.6$) (Fig. 6). The degree of mixing depends on the interplay between the rate of mantle plume upwelling and the rate of lithospheric plate divergence above the upwelling region, both of which are allowed to vary independently through time³³. Figure 6b compares the known temporal variation for the Iceland-Faeroe Ridge with the concurrent variation in spreading rate of the Mid-Atlantic Ridge, and a mantle plume flux, modified from Vogt and Avery's⁴⁹ curve for rate of basalt discharge in accordance with our new results. The (La/Sm) decline in the 50–60 Myr period could not be accounted for by a change of spreading rate as spreading would have had to increase rather than decrease³³. Therefore, the (La/Sm) decline must reflect a decrease in plume flux³³. Interpretation of the (La/Sm) secular decline within the past 13 Myr BP in Iceland depends on how reliable the spreading rate estimates are during this period (Fig. 6b)^{49,50}. At face value, the (La/Sm) decrease during the 9–13 Myr period could be accounted for by increasing spreading, but not during the past 9 Myr which again would require a decline in plume flux which would be particularly severe within the past million years. The blob model further suggests that we are witnessing in Iceland the declining phase of mantle blob activity, that is, a rapid change to a more passive mid-ocean ridge type spreading regime. If such a trend is extrapolated into the future, the model predicts either a change from a subaerial to a submarine regime and the possible split of Iceland into two islands located symmetrically about the Mid-Atlantic Ridge, or a new phase of plateau basalt formation, depending on the periodicity and topology of the proposed blobs.

It has been suggested²⁷ that the large (La/Sm) variation observed in successive flows sampled in DSDP Hole 336 on the northern flank of the Faeroes-Iceland Ridge⁵¹, may not support the proposed cycle (Fig. 6). We contend that what happens in the interim period between two blobs depends on the exact topology of the blob tail, that is the nature of schlierens trailing behind the main body of the blob (see ref. 52). Such interim periods may be characterized by inefficient mixing and thus possible alternation of lava with the geochemical signature of one source or the other. Rapid fluctuation between light-RE enriched and depleted patterns is also observed on the Reykjanes Peninsula^{25,37} as well as in the later phase of volcanism on the Faeroes just before the spreading began.

The blob-asthenosphere mixing model is supported by the detailed nature of the RE patterns shown in Fig. 2b. There is a (La/Sm) gap between <0.3 and 0.4 which corresponds to the field occupied by normal ridge segments adjacent to Iceland⁸. (La/Sm) variations on the right of this gap are explained by mixing the blob and depleted asthenospheric material whereas

to the left fractional melting is probably the cause of such unusually light-RE depleted picritic basalts which seem to occur only in Recent time, as previously suggested^{19,43,44}.

We further propose that the spatial variation in RE patterns and petrological basalt types found along the recognized neovolcanic and palaeorift zones of Iceland seems to depend on the degree of thermal and tensional tectonic maturity of the zones and existing mantle source heterogeneities beneath them. The northward progressive decrease in (La/Sm) and the increase in Mg-value observed along both the south-west and south-east zones, but at a faster rate in the south-east zone (Fig. 5), are best explained by a temporal change in intensity and conditions of melting resulting from rift propagation. For example, the south-west zone, a direct extension of the Mid-Atlantic Ridge exhibits high heat flow and well developed extensional tectonics¹ which we interpret as reflecting maturity¹⁹, whereas such features are lacking in the south-east¹, thus suggesting youth.

The thermal process resulting from such rift propagation is best described in two stages, though in reality it probably evolves more continuously. We assume first that crustal tension released at the tip of the rift propagator evolving along the south-east zone is sufficient to bring the older and colder late Miocene lower crust (~8 Myr old) into the partial melting stability field, resulting in magmas of crustal origin (and perhaps old lithosphere as well) with Sr and Nd isotopic signatures of late Miocene lavas exposed elsewhere on Iceland^{17,20,24}. RE patterns will also be light-RE enriched, but detailed modelling is required to take into account the possible mineralogical composition of the lower crust (and old lithosphere), degree of melting and type of lavas generated³¹. As spreading develops and the rift matures, there is a progressive change from primarily crustal-derived products to basaltic melts derived from mantle advection. We have seen earlier that the mantle source on Iceland in Recent time, particularly within the past 700,000 yr has become significantly diluted with light-RE depleted asthenospheric material. Thus, basalt erupted in a later stage, far beyond the tip of the rift propagator, will also be lower in ⁸⁷Sr/⁸⁶Sr, higher in ¹⁴³Nd/¹⁴⁴Nd (ref. 24), and less light-RE enriched. In other words, in this model the present spatial variation observed along the south-east zone is, in fact, the result of a temporal evolution in melting intensity and conditions, due to rift propagation. Melts erupting near the tip of the propagator are primarily derived from the older late Miocene lower crust-lithosphere, whereas in the northern part, as rifting and melting increase, basalts are derived from a mantle plume-asthenospheric mix, now dominated by the latter type of material. Increasing melting towards the centre of the plume (central Iceland) is supported by a concomitant increase in ²³⁰Th/²³²Th (ref. 53) and an increase in volcanic delivery rate which is manifested by a widening of the rift zones and a greater abundance of central volcanoes^{13,35,36}. The tip of the present rift propagator along the south-east zone probably lies just south of Surtesy Island, which emerged in 1963. Whether such a propagating rift is of the membrane type⁵⁴ as suggested for the Vestmannaeyjar-Surtsey Zone and the Settla Peninsula⁵⁵, or of Hey's type⁵⁶ is uncertain.

We also suggest that the south-west zone may also have developed by rift propagation, but earlier relative to the south-east zone, that is some 6 Myr BP (ref. 57). This is indicated by: (1) the similarity but different intensity of the gradient in (La/Sm) and Mg-value between the two zones (Fig. 5); (2) the presence of a well developed heat flow pattern and extensional tectonics in the mature south-west zone and their absence in the south-east zone¹; and (3) the presence of a ⁸⁷Sr/⁸⁶Sr gradient along the south-east zone but its absence in the more mature south-west zone (Fig. 5). Recent basalts erupted along the more mature south-west zone are all derived from the upper mantle composed of a blend of LILE-enriched blob and LILE-depleted asthenospheric material, with the degree of melting increasing slightly towards the centre of the plume (central Iceland) to account for the small northward decrease in (La/Sm) (Fig. 5)¹⁹.

If our assumptions concerning petrological and geochemical types and degrees of maturity of neovolcanic rift zones are correct and applicable to the more remote past, the following suggestion can be made on the nature of rifting throughout Iceland's history. Generation of the Eastern Plateau and the north-east zone lavas during the past 13 Myr seems to have been associated with continuous spreading and decreasing blob flux into the source region, and has been accompanied by flank rifting activity at least once in the past along the Skagi zone some 2.5–0.7 Myr BP. However, the petrological and geochemical uniformity of basalt erupted along the Skagi zone^{18,19} precludes that it developed by rift propagation. Thus, so far there is no evidence for rift propagation in northern Iceland. In contrast, the tectonic evolution of southern Iceland has been complicated by recurring southward propagation of rifts of ephemeral duration and simultaneous flank zone volcanism, as evident in the Borgarfjörður region (7–2 Myr), the south-east zone and the Setberg volcanic region on the Snaefellsnes Peninsula⁵⁸. This evolution is generally consistent with the tectonic evolution models proposed for Iceland^{12,14,57}. However, there are many uncertainties and several discrepancies which cannot be discussed further without more extensive field work in coordination with additional rock dating and geochemical and petrological studies.

Problems

Although our new data on Iceland support the blob model for the evolution of the Iceland–Faeroes hotspot, the genesis of such enriched blobs remains uncertain. The fact that such mantle material is enriched in the radioactive heat-producing elements (U, Th and K) could suggest a model by which internal heating of a deeper mantle layer rich in these elements would cause the layer to become gravitationally unstable and to rise in the form of blobs^{3,52}. Another possibility is the recent model involving lithosphere recycling⁵⁹. However, this is not supported by the high ³He/⁴He ratio of lavas near and on Iceland^{60,61}. The fact that such anomalous mantle regions are also richer in volatiles^{5,7,62} has also led to the suggestion that metasomatism of the upper mantle^{25,41,62,63}, perhaps resulting from fluids released from deeper mantle convection⁶², has produced the observed enrichments. In the latter case, interstitial fluids rising through the mantle and sweeping out large incompatible ele-

ments, accumulate in the upper mantle below the lithosphere. Such volatile enrichment would also enhance melting by lowering the melting point of the upper mantle and account for the greater volcanic flux in such regions. Application of this model to the Iceland–Faeroe Mid-Atlantic Ridge System would require that such a metasomatic front be geographically localized, spatially zoned, and episodic.

The above possible plume models are not necessarily exclusive. In fact, there is growing evidence that different plumes have different chemical and isotopic signatures and possibly different origins^{8,64}. Some may be related to lithosphere or crustal recycling; others may have a more primary origin^{60,61}.

Another point, inherent of the method of using lavas to constrain possible mantle flow geometries, is whether the gradients found along the Reykjanes Ridge^{2–4} reflect a similar zoning of the underlying mantle, or if they reflect a dynamical mixing condition of plume with depleted asthenospheric derived material during generation of new lithosphere². Distinction between these two possibilities would facilitate the choice between the plume or blob model and the metasomatic model. The difficulty lies in the fact that we do not know from what volume and geometry (topology) of the mantle a lava flow erupted on the Earth surface is derived from, has equilibrated with and thus is compositionally representative of. As an example of two possible extreme cases, we ask, is magma along the ridge axis derived primarily from a vertically deep and narrow column of mantle, or alternatively, laterally from a relatively thin mantle layer? Large lateral movement of magmas^{2,65,66} along strike during dyke propagation is also likely to add further interpretative complications.

Until these two major problems are resolved little advance can be made in choosing between the blob and metasomatic models, or other models including the veined mantle, or vertically zoned mantles of one type or another (see refs 67–69).

A more detailed discussion of the data can be found elsewhere^{31,70}.

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Processed genes: a dispersed human immunoglobulin gene bearing evidence of RNA-type processing

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A dispersed immunoglobulin pseudogene carries two hallmarks of RNA processing—spliced J and C regions and a poly (A)-rich tail. Its discovery strengthens the notion that processed genes are a significant feature of the mammalian genome and that genetic information can return to the genome via an RNA intermediate.

ONE of the α -like globin genes of the mouse has undergone two curious rearrangements; it has cleanly lost both its intervening sequences^{1,2} and it has moved from the active α -globin locus to another chromosome^{3,4}. This association of gene movement and precise splicing suggests that an RNA intermediate might have been involved in the formation of this novel gene^{1,2} as well as in its conveyance to a new location^{3,5}. The additional finding of multiple, distantly related members of the globin gene family³ further suggests that this gene is not an isolated example, but rather that dispersed and possibly spliced genes—or 'processed genes' as we shall refer to them—represent a significant class of genetic element and are the result of a major mechanism of genomic evolution. (By processed genes we refer to gene-like sequences that, as opposed to their normal counterparts, bear some evidence of RNA-type processing; for example, coincident homology to the site of transcriptional initiation, clean loss of intervening sequences or coincident homology to the site of poly(A) addition, possibly including elements of a poly(A) tail.)

Here we describe a human gene sequence that fulfills these expectations in a particularly interesting way. The gene is a pseudogene corresponding to the human immunoglobulin λ chain, the major locus of which is on human chromosome 22 (refs 6, 7) wherein the normal gene is represented in at least six non-allelic copies⁸. This pseudogene, $\lambda\psi 1$, is no longer at this locus, but has been conveyed to another chromosome. Here it is no longer represented as discontinuous J- and C-region segments, but rather the J and C regions are cleanly joined, again in accordance with the rules of RNA splicing. The gene bears an additional hallmark of RNA processing; its homology to the normal immunoglobulin gene ends abruptly in a long sequence of adenylic acid residues that resembles a poly(A) tail. These facts, in addition to the curious state of the 5' ends of this and the α -globin pseudogene, lead to a minimal—though entirely speculative—model that invokes an RNA intermediate in their formation and conveyance.

Identification and cloning of the λ -like genes

Our previous studies of the immunoglobulin λ light-chain genes of man had shown them to be arranged within a complex locus consisting of six constant region (and presumably at least an equal number of J region) genes, arrayed along a ~45-kilobase (kb) stretch of DNA on human chromosome 22 (ref. 8). When cleaved with the restriction endonuclease *EcoRI*, these genes fall into three fragments, usually 16, 14 and 8 kb long. The smallest (8-kb) fragment occasionally exists in one of several polymorphic forms, the most prevalent one in populations we have analysed being 18 kb. Several other more faintly

hybridizing fragments can also be identified by high-resolution gel electrophoretic analysis or by direct gene cloning. These include a large (>20 kb) fragment, an 18-kb fragment that underlies the strongly hybridizing 18-kb polymorphic fragment, a 16-kb fragment and a small 5-kb fragment that is evident in all human samples analysed. We have also cloned an additional, strongly hybridizing 14-kb fragment that has not yet been linked to the major locus.

The complexity of the human λ locus and its accompanying array of faintly cross-hybridizing gene fragments is reminiscent of the situation encountered among the mouse globin genes^{1-4,9-11}. The more distant relationship between the active λ genes and their structural relatives encoded on the >20- and 5-kb fragments is shown by high and low stringency in *in situ* hybridization experiments in which cloned active λ constant-region genes and a faintly hybridizing clone, $\lambda\psi 1$, are hybridized to total human genomic DNA (Fig. 1). When active gene sequences (genes 1 and 2 in Fig. 1, corresponding to the *Mcg* and *Kern⁻Oz⁻* genes) were used as probes, four strongly hybridizing bands were seen. Each corresponds to the four fragments that can be derived from the active locus of an individual polymorphic for both the 8- and 18-kb active fragments. In addition, faintly hybridizing >20- and 5-kb bands can be visualized in these high stringency conditions, and appear as more intensely hybridizing bands when the stringency of the washing conditions is reduced from 52 to 45 °C. The relative intensities of the >20- and 5-kb bands further suggest that the latter band is more closely related in sequence to the active genes than is the sequence encoded on the larger band (although it is also possible that the larger band is transferred less efficiently to the blotting paper or has a smaller segment of homology).

In any case, these blotting and cloning experiments, coupled with our inability to link the cross-hybridizing >20-, 18-, 16-, 14- and 5-kb fragments to the active locus through map extension strategies using cloned gene libraries (ref. 8 and P.H., G.H. and P.L., unpublished observations) raised the possibility that these fragments also encoded dispersed gene sequences. Therefore, we performed structural and gene mapping experiments on one of the most closely related of these, the 5-kb fragment referred to as $\psi 1$. It was cloned from purified genomic fragments of human lymphocyte DNA using the human λ constant-region probe in conditions of relaxed hybridization stringency (Fig. 1). Hybridization of the cloned, distantly related $\lambda\psi 1$ subfragment back to the genomic DNA exhibits the reciprocal pattern of fragments identified by the active λ gene probe (Fig. 1, lane d); that is, strong hybridization to the 5-kb fragment (itself) and weak hybridization to the active λ gene-containing fragments.

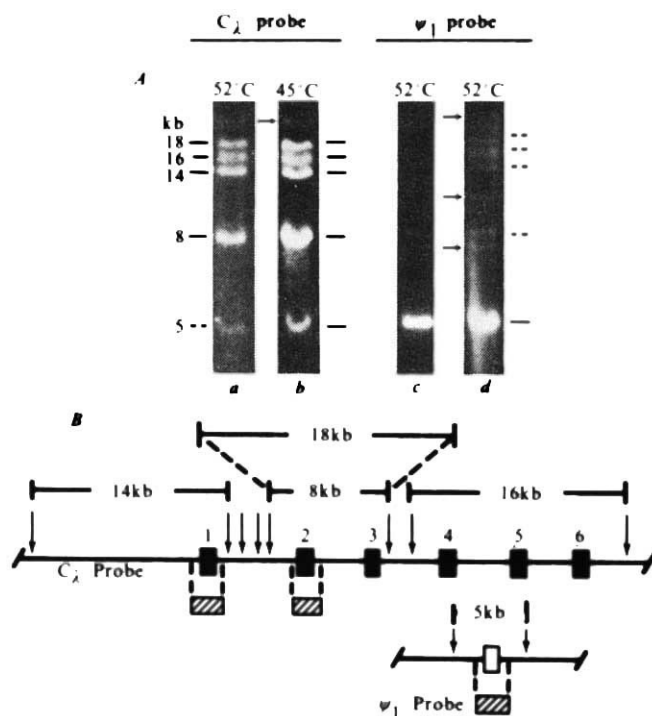


Fig. 1 Identification of cross-hybridizing DNA fragments related to human immunoglobulin λ genes. Genomic DNA was extracted from human spleen cells³², digested with *EcoRI* restriction endonuclease, size-fractionated by agarose gel electrophoresis (10 μ g per lane), transferred to nitrocellulose³³, hybridized with ³²P-labelled C_λ (a, b) or $\psi 1$ (d)³⁴, washed in 0.1 $\times$ SSC, 0.1% SDS at 52 $^\circ$ C (a, d) or 45 $^\circ$ C (b), and visualized by autoradiography. A control lane containing the cloned 5-kb *EcoRI* fragment is included (c). DNA fragments showing strong hybridization at 52 $^\circ$ C to the C_λ probe (a) are the 8-, 14-, 16- and 18-kb C_λ gene-bearing *EcoRI* fragments. In addition, a 5-kb *EcoRI* fragment hybridized weakly at 52 $^\circ$ C. When the stringency of the wash conditions was lowered to 45 $^\circ$ C (b), the 5-kb *EcoRI* fragment hybridization increased. This fragment was cloned and a 750-bp *PstI* fragment was shown to contain homology to C_λ . When this 750-bp fragment (see $\psi 1$ probe (B)) was hybridized to the cloned 5-kb *EcoRI* fragment (c) or human spleen DNA (d), a 5-kb *EcoRI* fragment hybridized strongly. The 8-, 14-, 16- and 18-kb C_λ gene-bearing fragments hybridized weakly (d) in these conditions. Four additional bands can be seen hybridizing very weakly and are indicated by arrows (see b and d). The C_λ probe (0.7-kb *EcoRI* fragment containing coding and flanking sequences of the human λ Mcg gene and a 3.4-kb *EcoRI*-*HindIII* fragment containing coding and flanking sequences of the λ Kern⁻Oz⁺ gene) and the $\psi 1$ probe (0.75-kb *PstI* fragment containing $\psi 1$ J-C processed gene) are shown in B.

Comparison of the active and pseudogene sequences

The region containing the λ -like sequence was subcloned from the $\lambda\psi 1$ hybrid phage and its nucleotide sequence determined according to the strategy shown in Fig. 2B. Similarly, the sequence of an active λ gene (gene 1, Fig. 1, Kern⁻Oz⁺) was determined for comparison. The two sequences (Fig. 2A) are extensively homologous over the region that corresponds to λ constant region (positions 108–540). Nevertheless, several insertions and deletions alter the $\lambda\psi 1$ sequence so as to create missense runs and termination codons, rendering it a pseudogene.

The points of divergence between the two genes are highly significant. On the 5' side of the sequence, homology ends at the border of the intervening sequence that would have been expected to separate C and J regions in a normal λ gene. A consensus RNA splice site, CCGCAG (PyPyNPyAG), present in the normal gene, is absent from the pseudogene. At the 3' side, homology ends abruptly 26 nucleotides to the 3' side of the putative poly(A) addition signal (AATAAA) that is conserved in both genes. In the pseudogene, the sequence has been replaced at each of these points of divergence by what seems to be evidence of RNA-type processing. The sequence on the 5' side closely resembles a J region complete with reasonable

5' V-J recombination signals spaced the required 12 bases apart^{12,13}. This homology to an authentic J region can be shown by comparing its nucleotide sequence with that of a known mouse J region (59% homology) and by comparing its translated amino acid sequence with that of a known human λ chain (Fig. 3). The sequence on the 3' side is replaced by a 33-base long poly(A) sequence interspersed with four C and two G residues. The comparable sequence in the active gene is T-rich. Also, a 9-base sequence occurs precisely at the termination of the poly(A) sequence (positions 673–681; Fig. 2A) that is repeated in the 5' portion of the processed gene (positions 15–23; Fig. 2A). This 9-base direct repeat may be the result of an insertion site duplication. In sum, the two sequences diverge from one another at sequences in the pseudogene that resemble those set in place by the two hallmark reactions of RNA processing, the splicing of coding sequences and the addition of poly(A) tails.

Although the pseudogene closely resembles a processed mRNA rather than an intact gene in these important respects, it differs from a normal mRNA sequence in one important way. An active immunoglobulin gene is formed by the joining of a germ-line V region to a germ-line J sequence^{12–14}. In a few examples thus far studied, the site of transcriptional initiation of these active genes is provided by the incoming V region. Thus, the normal transcriptional initiation site is ~30 nucleotides 5' from the beginning of the V-region coding sequence¹⁵. The pseudogene sequence noted above completely lacks a joined V-region sequence and indeed retains a reasonable copy of a V-J (DNA) joining signal. Therefore, its similarity to a normal mRNA breaks down in that it lacks the 5' portion of a normal λ mRNA, the V region, and hence lacks homology to a normal immunoglobulin promoter. We shall return to this problem below.

The processed pseudogene is also dispersed

The initial example of what we refer to as a processed gene, the mouse $\alpha\psi 1$ sequence, resides on mouse chromosome 15, whereas the active mouse α -globin locus is on chromosome 11 (refs 3, 4). Evidently, this processed gene must have been conveyed by some transpositional mechanism from the major globin locus. As gene movement might have a significant role in the formation of processed genes, it was obviously of great importance to determine whether or not the processed λ sequence could be linked to the main λ locus on chromosome 22 (ref. 6). The relationship of these genes was determined in mouse $\times$ human hybrid cell lines that retain different human chromosomes by detecting characteristic restriction endonuclease fragments corresponding to each gene in the various hybrid cell lines¹⁶.

Figure 4 gives an example of such an analysis. Cell line 2 retains the active genes as well as the processed gene, while cell line 1 retains only the active genes. In contrast, cell line 8 retains the pseudogene, but lacks the active genes. As cell lines 1 and 2 retain chromosomes 22 and the cell line 8 does not, these results are consistent with the absence of the processed gene from the normal λ locus on chromosome 22. Similar studies on 24 hybrid cell lines are consistent with this conclusion (λ^+ , $\psi\lambda^+$, three cell lines; λ^- , $\psi\lambda^+$, six cell lines; λ^+ , $\psi\lambda^-$, three cell lines; λ^- , $\psi\lambda^-$, 12 cell lines; data not shown), but they do not yet allow us to specify unambiguously the chromosomal location of the processed gene.

Evidence of RNA-type processing

The spliceless α -globin pseudogene initially suggests that its formation might have been mediated by its processed RNA transcript^{1,2}, and this is supported in several important respects by the discovery of the immunoglobulin processed gene described here. First, the λ -processed gene also bears evidence of two RNA processing events; it has lost its intervening sequences in accordance with the rule of RNA splicing and, like the globin processed gene, its homology with its normal counterpart ends abruptly at a poly(A) addition site. However, unlike the

globin processed gene, its sequence at this point is joined to an extensive poly(A) region (Fig. 2). These features support the notion that processed RNA transcripts were involved in the formation of both genes.

But what of the 5' ends of the two processed genes? Neither of these resembles that of its normal mRNA analogue. The globin sequence homology extends beyond the site of normal transcription initiation and the immunoglobulin sequence clearly has no normal counterpart to the 5' portion of an active λ mRNA. It is therefore clear—if these processed genes were formed from RNA transcripts—that they were aberrantly initiated. (There is some evidence for the formation of such aberrant transcripts in globin and immunoglobulin-producing cells¹⁷⁻¹⁹.) If this is indeed the case, it follows that this 'aberrant' transcript contains genetic information normally excluded from mature mRNAs.

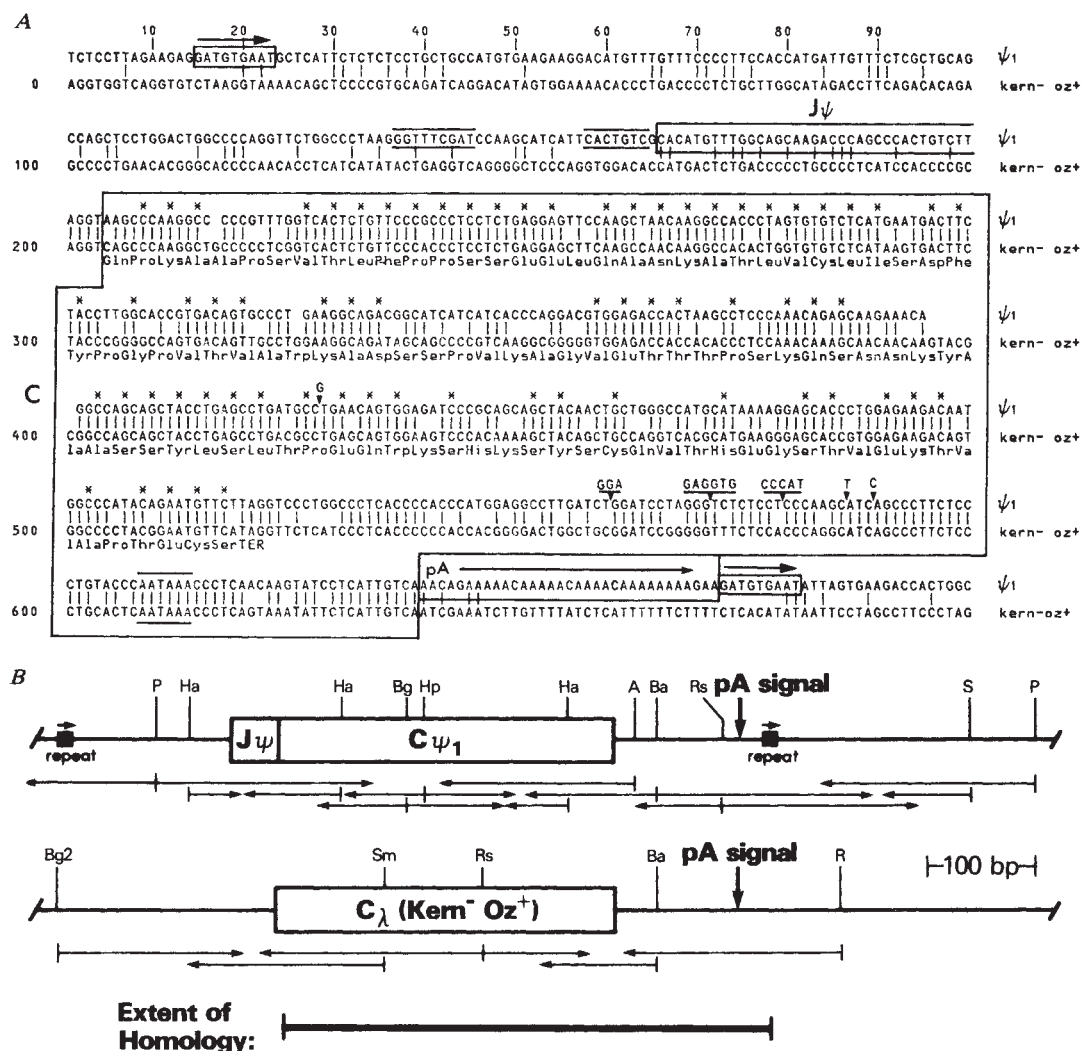
Potential mechanisms for processed gene formation and conveyance

Goff *et al.*⁵ have noted several useful features of the retroviruses that might account for the conveyance and structure of processed genes. A genomic fragment of DNA, once incorporated into a retrovirus sequence, could be processed so as to lose its intervening sequences during the RNA phase of the viral life cycle. Indeed, there are several examples of incorporate host genes that are represented in spliced form in the retrovirus^{8,20}. However, for such a vector to have incorporated one

of the processed globin or immunoglobulin genes, we would have to postulate a special mechanism to explain how the sequence homology of the processed genes ends at the poly(A) addition site. This feature suggests that the sequence would have to have been taken up as a processed RNA rather than first incorporated, unprocessed, into the retroviral sequence. While this does present a difficulty, gene mapping studies indicate that two retrovirus-like sequences flank the processed α -globin gene, although their configuration does not accommodate a simple model for the role, if any, a retrovirus may have had in their formation²¹.

Two other classes of genomic sequence have also recently been implicated in the formation of processed genes. These are multi-gene families that correspond to the middle repetitive *Alu* genes²² and the multiple snRNA genes that encode the ubiquitous small nuclear RNAs prevalent in human (and other) cells²³. Members of both gene families are transcribed into small RNAs of unknown function²⁴⁻²⁷, the *Alu* sequence apparently in response to RNA polymerase III²⁸, the snRNA sequence in response to RNA polymerase II²⁹. Although there are multiple active copies of both gene sequences, recent structural studies suggest that many members of these repeated families are pseudogenes, that is, genes that differ from the major expressed version of the sequence^{23,24}. In addition, several features of these genes are similar to integrated transposons: they are flanked by short direct repeat sequences reminiscent of target site duplications, and they are dispersed in the sense that they do not occur in large tandem arrays. (A curious 3.6-kb insertion

Fig. 2 A, Comparison of the sequences of the human λ *Kern⁻Oz⁺* and processed λ gene, $\psi 1$. The nucleotide sequence of λ *Kern⁻Oz⁺* is presented as a reference sequence and a limited number of gaps and insertions have been introduced into the $\psi 1$ sequence to maximize homology. The amino acid sequences deduced from the gene sequence (*Kern⁻Oz⁺*)³⁵ are shown and an asterisk above the *Cp1* sequence indicates that the amino acid position is identical to the λ sequence (*Kern⁻Oz⁺*). The extent of homology corresponds to the sequence boxed and labelled C and covers nucleotide positions 205–638. At the 5' end of the λ gene there is a consensus



BglII, Ks, KsaI, S, SalSA,
Bg2, BglII; Sm, SmaI; R, EcoRI. ³²P-labelled ends are represented by short vertical lines and the extent and direction of sequencing are indicated by arrows. The boxed area of CA (*Kern-Oz'*) indicates the coding region (amino acids 108–215 of λ polypeptide) and 3'-untranslated region of the mRNA. The boxed area of ψ1 indicates the sequence that corresponds to J- and C-type sequences. Extent of homology between these genes is shown by a bold black line.

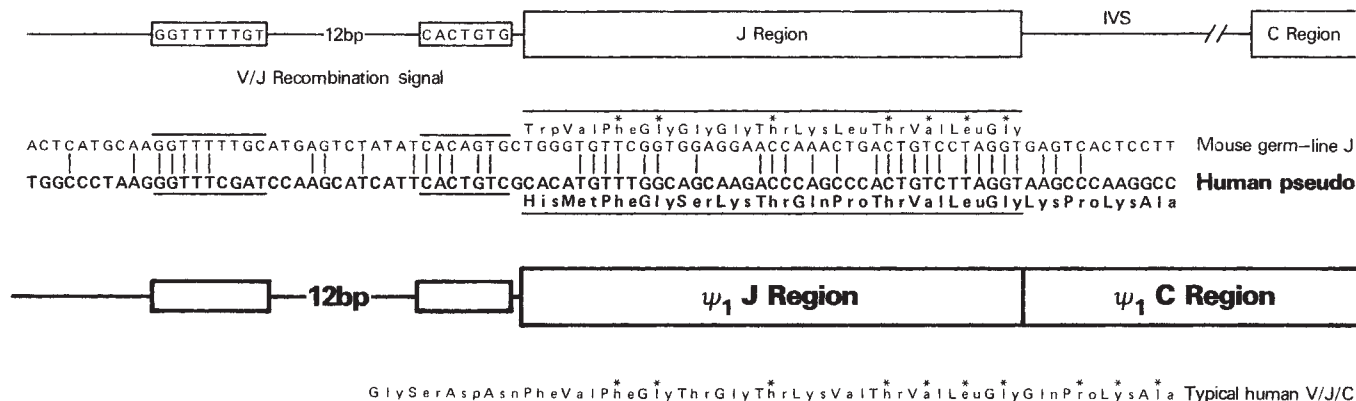


Fig. 3 Comparison of the J-like region of the processed λ gene, $\psi 1$, to a germ-line mouse λ J-region sequence and a typical human J-region amino acid sequence. The nucleotide sequence of $\psi 1$ J region is compared with the nucleotide sequence of a mouse germ-line J region³⁷. The amino acid sequences deduced are shown above and below the nucleotide sequences and an asterisk above the mouse germ-line J amino acid indicates that the amino acid position is identical to the human $\psi 1$. A typical human V-J-C sequence determined at the protein level³⁸ is also shown and an asterisk above the human protein sequence indicates that the amino acid position is identical to the human $\lambda 1$. In addition to the homology seen in the J coding region, the human $\psi 1$ contains remnants of the V-J recombination signal seen in the germ-line sequence of the mouse (indicated by horizontal lines above and below the nucleotide sequence). The homology between the mouse germ-line J and human $\psi 1$ breaks down 3' to the J region, an area where the mouse sequence corresponds to intervening sequence, while the human $\psi 1$ is homologous to CA sequence.

has been found in a *Drosophila* ribosomal gene that is bracketed by 13-base pair (bp) direct repeats³⁰. Careful review of this sequence shows it to end in an A-rich sequence near a poly(A) addition signal.) An additional feature of the two former classes of pseudogene is that their 5' ends, unlike the processed genes we have described, coincide with the normal transcription initiation sites of their RNAs. This fact, and the fact that some, but not all, of these pseudogenes terminate in a poly(A) sequence, have led two groups^{23,24} to propose that they represent a class of transposable element that passes through an RNA intermediate.

Keeping these more detailed proposals in mind, we will consider a minimalist model for the formation of the globin and immunoglobulin processed genes (Fig. 5). First imagine that these processed genes arise from aberrant transcripts that begin at some distance 5' to the genes' normal initiation sites. These transcripts are processed, coding sequences are spliced and poly(A) added. The RNA is converted to DNA and then re-integrated into chromosomal DNA. It is also conceivable that a special sequence has been incorporated into the processed transcript that is necessary for its integration, for example, a retrovirus sequence as suggested by Goff *et al.*⁵, or an *Alu* or *snRNA* element as suggested by Van Arsdell *et al.*²³ and

Jagadeeswaran²², or some other, undiscovered, sequence that shares the mobility properties tentatively ascribed to these elements. Such mobility elements would not normally be transcribed into mRNAs because they lie beyond the usual transcription boundaries of an active gene. Remember that these hypothetical events must have occurred in a germ cell where transcription boundaries and gene activities may differ dramatically from those found in terminally differentiated cells.

If integration of a processed gene does not require a specific integration element and is a fundamental property of any RNA or its cDNA cognate, processed genes might arise from any transcribed sequence. If so, we would expect eventually to find a processed gene that corresponds precisely at both 5' and 3' ends to a normal mRNA. On the other hand, if a mobility element is required, we might expect to find copies of the mobility element still encoded near the parental locus and homologous to a sequence that has been transposed with the processed gene. We might also expect this element to be present near other processed genes. Finally, since we might expect that such sequences would remain a source of these processed genes for a long period of evolutionary time, we might expect to find further examples of processed genes that originate from the

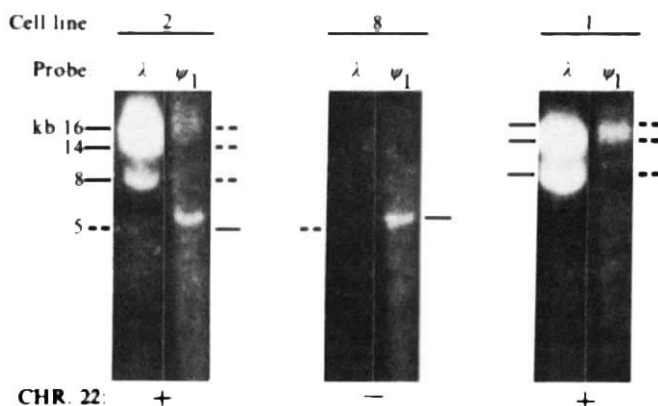


Fig. 4 Discordance of normal and processed λ genes in mouse $\times$ human cell hybrids. DNA isolated from somatic hybrid cell lines was digested with *EcoRI*, fractionated by agarose gel electrophoresis (30 μ g per lane), transferred to nitrocellulose³³, hybridized with a human CA probe or $\psi 1$ probe (see Fig. 1)³⁴ and visualized by autoradiography. Human chromosome 22 carries the active human λ gene locus and is present in cell lines 2 and 1 (ref. 6). The 5-kb *EcoRI* fragment containing $\psi 1$ gene segregates discordantly with chromosome 22 because it appears in cell line 8 (a cell line that does not contain chromosome 22) and does not appear in cell line 1 (a cell line that does contain chromosome 22).

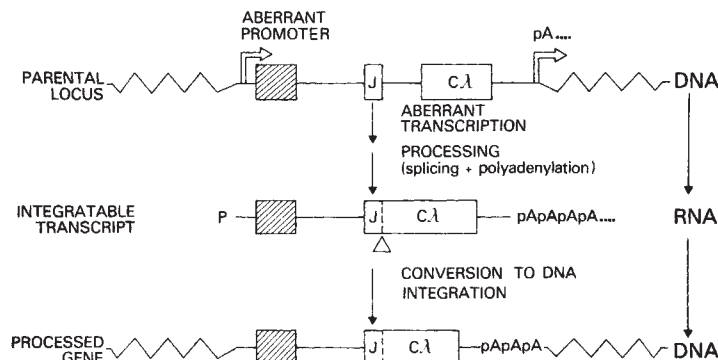


Fig. 5 A minimal model for the formation of the processed CA gene. The top line represents the germ-line configuration of the putative ancestral CA gene, showing (in boxes) the J and C coding regions as well as the site of a proposed aberrant promoter (arrow) and of poly(A) addition (arrow). The hatched box represents a hypothetical sequence that might render a transcript of this region integratable, a detail that is not required if we assume that any RNA sequence (or its cDNA cognate) can ultimately be integrated into chromosomal DNA. The proposed scheme simply calls for an aberrant transcript of this sequence to arise in a germ cell, be processed as an RNA (spliced and polyadenylated), then be converted into DNA for subsequent (or simultaneous) incorporation into a new chromosomal location. Note that information flows from DNA to RNA back to DNA.

affected member of the active gene family. We already know that there are additional, unlinked copies of both genes that are processed gene candidates. It is therefore possible that processed genes represent a major class of genomic element and, while several mechanisms are available for their conveyance, their structures strongly support the notion that RNA transcripts have been involved in their formation. Consistent with this notion, N. Cowan and colleagues (personal communication) have discovered an interesting human α -tubulin-like

pseudogene that has lost its intervening sequences and ends in a poly(A) tail. We expect many such processed genes will be discovered as other multi-gene families are examined in greater detail. Thus, the notion that information can flow from DNA to RNA back to DNA, long established in the case of the retroviruses³¹, seems to involve non-viral genes as well.

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Immunoglobulin heavy-chain expression and class switching in a murine leukaemia cell line

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A cell line that switches from μ to $\gamma 2b$ synthesis during growth in culture uses the same V_H region for both heavy chains but retains two copies of the C_μ gene. This suggests that the μ to $\gamma 2b$ class switch can occur, at least in part, by an RNA processing mechanism. Regulatory variants of this cell line lose constitutive μ -chain synthesis but simultaneously acquire lipopolysaccharide(LPS)-inducible synthesis of that chain. This co-variation is allele-specific and is correlated to a large deletion of DNA in the J_H - C_μ intron.

INFECTION of murine bone marrow or fetal liver cells with Abelson murine leukaemia virus (A-MuLV) transforms a small fraction of the cells into clonal, continuous cell lines¹. Various properties of these lines indicate that they are generated from immature cells of the B-lymphoid (immunoglobulin-producing) cell lineage, the majority being related to the most immature cell known to occur in this pathway, the 'pre-B' cell^{2–4}. Surveys of the structure and expression of immunoglobulin genes in large numbers of such lines have proved extremely useful for defining the pre-B stage of differentiation at a molecular level⁴. In addition, these studies have defined specific subclasses of A-MuLV transformants which exhibit individual aspects of the immunodifferentiation programme. One such cell line is 18-8, and its clonal derivative 18-81, which usually produces μ chains in the absence of detectable light chains² but from which certain clonal isolates also produce κ chains after stimulation with bacterial lipopolysaccharide (LPS). The latter phenomenon is probably due to productive κ -gene rearrangement during growth of the 18-8 line in culture^{5,6}. Synthesis of γ -related

chains has also been detected in populations of these cells² and in subclones of these lines⁷. The work of Burrows *et al.*⁷, together with our preliminary studies⁵, suggested that during growth in culture, the 18-81 cell line may undergo heavy-chain class switching; this is the process by which a clone of B-lymphoid cells first expresses a single heavy-chain variable (V) region in association with μ heavy chains and subsequently with another heavy-chain class such as α or γ (ref. 8). The process often appears to be effected by a recombination/deletion mechanism. The order of the C_H genes has been determined as 5'-($V_H J_H$)- C_μ - C_δ - $C_{\gamma 3}$ - $C_{\gamma 1}$ - $C_{\gamma 2b}$ - $C_{\gamma 2a}$ - C_ϵ - C_α -3' (ref. 9) so that, for example, expression of $\gamma 2b$ protein in a myeloma would result from juxtaposition of the $C_{\gamma 2b}$ gene with the expressed $V_H J_H$ complex by deletion of the intervening DNA sequence, including the C_μ , C_δ , $C_{\gamma 3}$, and $C_{\gamma 1}$ genes^{10,11}. Most of the evidence for the deletional model of heavy-chain class switching has come from comparative studies of heavy-chain gene structure in independently derived immunoglobulin-secreting myelomas^{9–16}. Clearly, cloned cell lines such as 18-8, which undergo class-switching in culture, could be useful for elucidating this process.

In addition, certain properties of 18-8 and its sublines suggest that these cells may be useful for generating variants having

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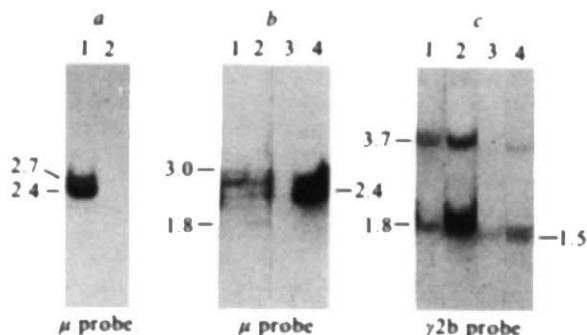


Fig. 1 Detection of μ and $\gamma 2b$ RNA sequences in various 18-81 isolates. The derivation of the 18-8 line and its clonal derivative 18-81 has been described previously². Subclones of 18-81 were derived by dilution and plating in microtitre wells at <1 cell per well. For LPS treatment, cells were plated at 4×10^5 cells ml^{-1} and grown in the presence of $100 \mu\text{g ml}^{-1}$ *Salmonella typhimurium* LPS (Difco) for 48 h^{17,34}. Parallel control cultures were evaluated in each experiment. To examine the RNA in the various 18-81 cultures, $\sim 10 \mu\text{g}$ of total poly(A)-containing RNA prepared from the indicated sources was fractionated by electrophoresis through methylmercury hydroxide agarose gels, transferred to diazotized paper and hybridized with $\sim 10^7$ c.p.m. of a nick-translated cloned DNA probe for either μ sequences (pAB μ -1) or $\gamma 2b$ sequences (pAB $\gamma 2b$ -1) (specific activity between 10^8 and 10^9 c.p.m. μg^{-1}). The pAB μ -1 plasmid contains a 770-base pair (bp) μ cDNA insert¹⁸ and the pAB $\gamma 2b$ -1 a 1,200-bp $\gamma 2b$ cDNA insert⁴⁰. *a*, μ probe, RNA from: lane 1, young 18-81 culture; lane 2, 18-81A. *b*, μ probe, RNA from: lanes 1 and 2, 81A-2 cells; lanes 3 and 4, 81A-20 cells. Cells were either untreated (lanes 1 and 3) or treated with LPS (lanes 2 and 4). *c*, $\gamma 2b$ probe: lanes as indicated for *b*.

altered regulation of immunoglobulin synthesis. During propagation of the 18-81 line in culture, its relatively high level of μ -chain synthesis (0.1% of soluble protein) decreases; after 9–12 months of culture it makes $<10\%$ of the μ chain found in the parent population¹⁷. The synthesis of μ chains in long-term cultures of 18-81 can be increased to a level approaching that of fresh cultures by treatment of the cells with bacterial LPS¹⁷. This suggests that growth of 18-8 cells in culture may select for variants having stable changes in the regulation of immunoglobulin heavy-chain gene expression. Such variants would be invaluable for studying the molecular basis of the various transitions in the level of heavy-chain gene expression that occur during B-cell differentiation.

Here we characterize cloned variants of the 18-8 line which exhibit one or both of the properties described above. The studies indicate that in these variants, heavy-chain class switching may occur without deletion of the C_μ gene and that the genetic alteration leading to decreased heavy-chain expression (and apparently the simultaneous acquisition of LPS inducibility of expression) is linked to DNA alterations near the functionally expressed V_H allele.

Loss of μ production during culture correlates with decreased levels of μ mRNA

To characterize the events involved in the decrease of μ protein synthesis in derivatives of cell line 18-8, we first analysed the relative level of μ -related RNA sequences in a line cloned soon after the establishment of 18-8 (called 18-81) and in the same line after 9 months of continuous passage (called 18-81A). Line 18-81 produced $\sim 0.1\%$ of its protein as μ chains; 18-81A had no detectable μ chains. For these analyses, total poly(A)-containing RNA was prepared, fractionated, transferred to diazotized paper and assayed for hybridization to ^{32}P -labelled μ -specific cloned DNA probes as described previously¹⁸. Hybridization with a probe specific for C_μ revealed a prominent 2.4-kilobase (kb) μ RNA, as well as a minor 2.7-kb species in the young culture (Fig. 1*a*, lane 1) but no detectable μ RNA in the long-term culture (Fig. 1*a*, lane 2). Hybridization with

sub-probes specific for μ_m or μ_s domains demonstrated that the 2.4- and 2.7-kb μ RNAs in the parent populations corresponded respectively to μ_s and μ_m RNA (ref. 18). Therefore, the decrease in μ protein production that occurred when the 18-81 line was grown in culture correlated with a reduction in μ mRNA accumulation.

Subclones of 18-81A produce either LPS-induced μ or $\gamma 2b$ protein and mRNA

After ~ 9 months in culture the 18-81A line was subcloned to produce the 81A series of cell lines. Individual 81A lines were then examined for heavy-chain production by metabolic labelling and specific immunoprecipitation. Consistent with the behaviour of the parental line, of 40 81A lines examined, none constitutively synthesized significant levels of μ -related proteins. However, all but one of the lines synthesized μ protein after treatment with LPS; the behaviour of a representative line, 81A-20, is shown in Fig. 2, lanes 1 and 2. One line, 81A-2, produced no μ with or without LPS treatment but produced detectable levels of a γ chain in the absence of LPS treatment (Fig. 2, lane 3) and greatly increased amounts after LPS treatment (Fig. 2, lane 4). Analyses using sera specific for various γ classes indicated that the γ produced by 81A-2 was $\gamma 2b$ (not shown). This line also produced low levels of a μ -sized chain that was precipitated in our assay conditions (Fig. 2, lane 4), but which does not appear to represent μ protein because: (1) the precipitation of this protein occurred at variable levels; (2) it was observed with *Staphylococcus aureus* treatment alone; (3) it was not observed with double antibody precipitation; and (4) its precipitation was not competed with authentic μ protein (data not shown).

To ascertain that the γ -producing 81A-2 clone, the μ -producing 81A-20 clone and the 18-81 line were all sibling clones, we examined their integrated A-MuLV proviruses⁵. All the clones had identically integrated proviruses⁵, and must therefore have been descendants of the same, initially transformed cell because individual A-MuLV transformants have distinguishable proviruses¹⁹.

Analyses of the RNAs produced by the 81A lines yielded results parallel to those obtained by protein analyses. The μ -inducible, 81A-20 line contained a barely detectable level

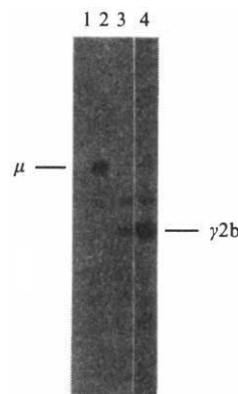


Fig. 2 LPS enhancement of μ and $\gamma 2b$ production in various 18-81 isolates. Cell lines 81A-20 and 81A-2 were treated with LPS as described in Fig. 1 legend, then metabolically labelled with ^{35}S -methionine ($80 \mu\text{Ci ml}^{-1}$) for 60 min as previously described^{2,18,41,42}. Control cultures which did not receive LPS were incubated and labelled in parallel. Extracts were then prepared and incorporation into μ - or γ -related polypeptides determined by precipitation with rabbit anti-mouse immunoglobulin and SDS-polyacrylamide gel electrophoresis of the resulting precipitates as previously described^{2,18,41,23}. Lane 1, 81A-20, no treatment; lane 2, 81A-20, LPS; lane 3, 81A-2, no treatment; lane 4, 81A-2, LPS. Identity of μ and γ chains was confirmed by specific immunoprecipitation and competition assays as previously described¹⁸.

of 2.4-kb μ_s mRNA which was greatly augmented by LPS treatment (Fig. 1b, lanes 3 and 4). A 1.5-kb γ -related RNA, the level of which was not significantly affected by LPS treatment, was also evident in this line when the same RNA was assayed using a γ 2b probe (Fig. 1c, lanes 3 and 4). This RNA species, however, does not appear to contain a V_H sequence (see Fig. 4) and is probably related to the 'sterile μ ' RNA transcripts described below.

The γ 2b-inducible 81A-2 line produced a diffuse pattern of μ RNA species ranging in size from 3.0 to 1.8 kb and the abundance of these species was not affected by LPS (Fig. 1b, lanes 1 and 2). These 'sterile μ ' RNA sequences²⁰, which are probably related to those described by others²¹, are found in many A-MuLV-transformed cell lines and do not encode μ chains²⁰. The 81A-2 line also contained 1.8- and 3.7-kb species of γ -related RNA (Fig. 1c, lane 1); the level of the 1.8-kb species was greatly augmented by LPS treatment (Fig. 1c, lane 2). Characterization of these RNAs with DNA probes specific for the various γ subclasses confirmed their identity as mRNAs for the membrane-bound (3.7 kb) and secreted (1.8 kb) forms of γ 2b protein^{22,23}. Thus, as observed by analyses of both protein and RNA, clone 81A-2 has apparently stably shifted from μ to γ 2b production in culture. In addition, like μ expression in the parental line or sibling clones, γ 2b expression is low in 81A-2 but inducible to high levels by treatment of the cells with LPS.

Heavy-chain gene rearrangements in the 18-8 series

To examine whether the switch to γ 2b production in 81A-2 was paralleled by changes in the cell DNA, we performed a

restriction endonuclease analysis of the C_μ and J_H regions in the DNA of 81A-20 and 81A-2. In the germ line of the BALB/c mouse, all the C_μ exons occur on a single *Eco*RI fragment of 12.5 kb, called here the RI- C_μ fragment, and the J_H segments are located on a contiguous 6.2-kb *Eco*RI fragment, called here the RI- J_H fragment (Fig. 3). Any rearrangement in which a V_H or D_H region is abutted to a J_H should abolish the germ-line RI- J_H fragment and generate a new fragment containing the DNA sequence lying between the active J_H and the nearest 3' *Eco*RI site.

To assay the DNA segments in various cell lines, DNA was prepared, digested with *Eco*RI and assayed for hybridization to ³²P-labelled DNA probes representing either the RI- J_H fragment⁵ or the C_μ region¹⁸. As observed with embryo DNA (Fig. 3a, lane 1), a major, 12.5-kb *Eco*RI fragment in the DNA of the 18-8 parental line hybridized to the C_μ probe (Fig. 3a, lane 2). When assayed with the RI- J_H probe, however, two fragments of ~2.4 and 1.8 kb were observed in the parental 18-8 line (Fig. 3b, lane 2), neither of which corresponded to the unrearranged 6.2-kb fragment observed in DNA from mouse embryo (Fig. 3b, lane 1). This result indicates that both J_H alleles had undergone rearrangement in the 18-8 line and was confirmed by demonstrating that the 1.6-kb *Eco*RI fragment directly 5' to the RI- J_H fragment in embryonic DNA was absent from the genome of 18-8 and its daughter lines⁴.

In the DNA of the 18-81A line, only a single *Eco*RI fragment of 8.0 kb hybridized to the C_μ probe (Fig. 3a, lane 3). In addition, this cell population contained only a single observable RI- J_H fragment which was identical in size (8.0 kb) to that containing the C_μ gene (Fig. 3b, lane 3); this was confirmed by eluting the RI- J_H probe and rehybridizing with the C_μ probe (data not shown). These results imply that one J_H - C_μ allele had been lost

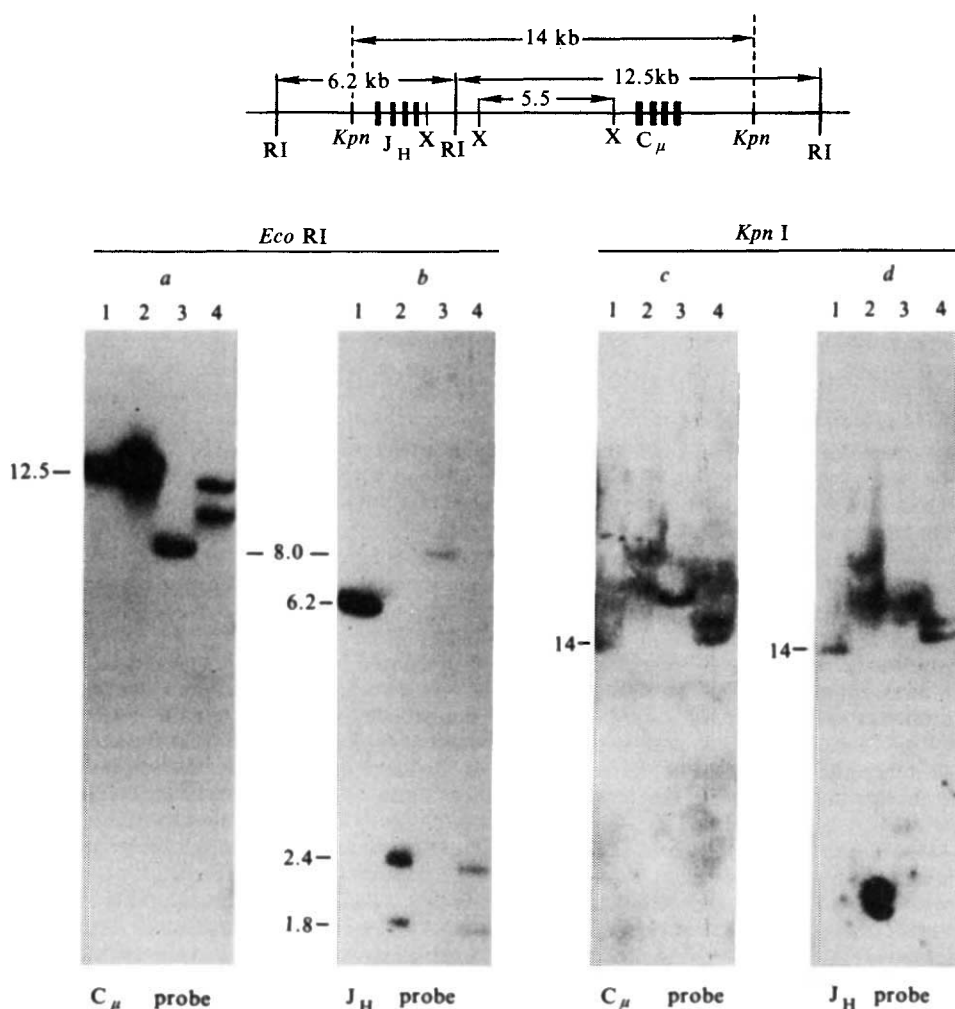


Fig. 3 Analysis of heavy-chain gene rearrangements in various 18-8 isolates. Upper part, restriction map of J_H - C_μ region. Relevant *Eco*RI (RI), *Kpn*I (*Kpn*) and *Xba*I (*X*) sites are indicated. Lower part, approximately 10 μ g of cellular DNA from indicated sources were digested with *Eco*RI (panels a, b) or *Kpn*I (panels c, d) and assayed by DNA blotting procedures for hybridization to the ³²P-labelled (final specific activity = 10^8 – 10^9 c.p.m. μ g⁻¹) pAB μ -1, C_μ probe (panels a, c) or the pRI- J_H , J_H probe (panels b, d) as described elsewhere⁴. Plasmid pAB μ -1 is described in Fig. 1 legend; pRI- J_H has a 6.2-kb insert which corresponds to the embryonic *Eco*RI fragment containing the J_H segments (see map above). Lane 1, BALB/c embryo DNA; lane 2, 18-8 DNA; lane 3, 18-81A DNA; lane 4, 81A-2 DNA.

in the 18-81A population whereas the other had undergone a deletion of ~5-6 kb spanning the *Eco*RI site between RI-J_H and RI-C_μ.

The location of the deletion in the single J_H-C_μ region in line 18-81A was also confirmed by hybridization with sub-probes specific for this intron region (see below) and by cleavage analyses with other enzymes. An identical 8.0-kb fragment hybridizing to both the RI-C_μ and RI-J_H probes was also observed in the DNA of 81A-20, the μ-inducible clone (data not shown), implying that the heavy-chain allele containing the large deletion—the only one present—is the one expressed in this and therefore the other 18-8 related lines. In addition, a loss of one C_μ allele and a deletion in the other occurred when a separate population of 18-81 was maintained in culture until μ-gene expression substantially decreased (data not shown).

Analysis of the RI-J_H region in the DNA of the γ2b-producing derivative, 81A-2, indicated the presence of RI-J_H regions originating from both chromosomes and showed no evidence of rearrangement with respect to the configuration found in the 18-8 ancestral line (that is, 2.4- and 1.8-kb fragments; Fig. 3b, lane 4). In addition, two RI-C_μ fragments of 11.0 and 9.5 kb were found in the DNA of this line (Fig. 3a, lane 4). This result suggested that the shift to γ2b production in 81A-2 may have occurred without deletion of a C_μ gene.

To examine further whether 81A-2 retained both C_μ alleles, we analysed the J_H- and C_μ-containing fragments generated when the DNA of this line was digested with *Kpn*I. Figure 3 shows that *Kpn*I cuts 3' to the C_μ exons and 5' to the J_H segments, thus generating a single 14-kb embryonic DNA fragment containing both these regions. Rearranged genes, in which the C_μ regions are still associated with the J_H region, should result in identical patterns of hybridization when *Kpn*I-digested DNA is assayed with either RI-J_H or C_μ probes. Juxtaposition of the J_H region to a γ2b allele, however, might be expected to produce a unique *Kpn*I fragment when assayed with RI-J_H. As predicted, C_μ and RI-J_H probes showed identical patterns of hybridization to a 14-kb *Kpn*I fragment of embryonic DNA (Fig. 3c, d, lanes 1) (the C_μ-containing fragment was more distinct in other experiments). In DNA from line 18-8, both probes hybridized to 18.5- and 17-kb fragments (Fig. 3c, d, lanes 2). In DNA from 81A-20, the probes located only a single 17-kb fragment, consistent with the presence of only one J_H-C_μ region (Fig. 3c, d, lanes 3). In DNA from line 81A-2, two fragments of 16 and 15 kb hybridized to both probes (Fig. 3c, d, lanes 4), suggesting that both J_H alleles were still associated with C_μ genes in the γ2b-producing line.

The same V_H-J_H complex is first expressed with μ and then with γ2b

To prove rigorously that a switch had actually occurred during the propagation of the 18-81 cell line, we examined whether the same V_H gene expressed with C_μ in the parental 18-81 line was subsequently expressed in association with C_{γ2b} in the 81A-2 subclone. We molecularly cloned both RI-J_H fragments from 81A-2 into λ phage Charon 16A and demonstrated by nucleic acid sequencing that they contained independently rearranged V_H genes rearranged to J_H2 (V_H-81X) and J_H3 (V_H-81Y) (structures summarized in Fig. 4; data to be presented elsewhere). From these two clones we prepared the two V_H-specific probes as indicated in Fig. 4, and these were then labelled with ³²P by nick-translation and used to probe total poly(A)-containing RNA isolated from lines 18-81A, 81A-2 and 81A-20 both with and without (not shown) LPS treatment.

V_H-81Y hybridized to 2.7- and 2.4-kb RNA species in both 18-81A (not shown) and 81A-20 RNA (Fig. 4a, lane 1) with a pattern identical to that obtained when these RNAs were assayed with a C_μ probe (Fig. 1a, lane 1; Fig. 1b, lane 4). Furthermore, this probe hybridized to 1.8- and 3.7-kb γ RNA species in 81A-2 RNA (Fig. 4a, lane 2), a result identical to that obtained when this RNA sample was assayed with a γ2b probe (Fig. 1c, lane 2). The V_H-81X probe did not hybridize

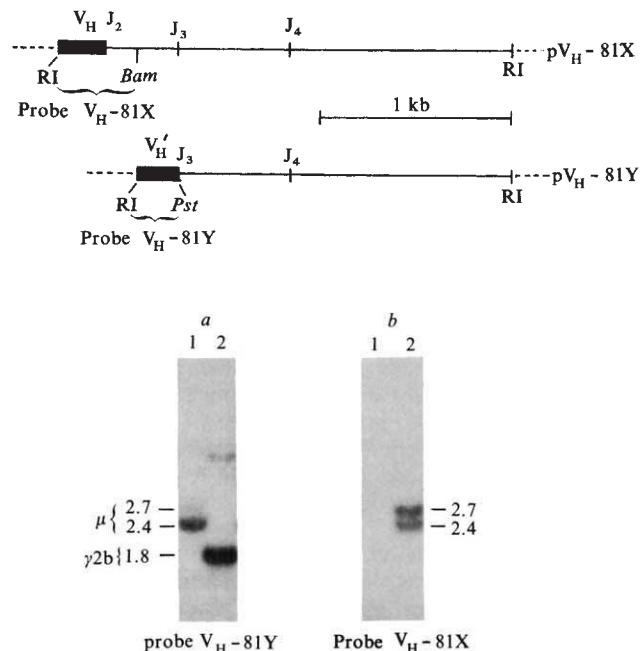


Fig. 4 Upper part, partial restriction map of clones pV_H-81X and pV_H-81Y. The plasmids pV_H-81X and pV_H-81Y have, respectively, 2.4- and 1.8-kb inserts containing the rearranged V_HJ_H complexes from 81A-2 as indicated in the map. The plasmids were derived by subcloning the inserts from *Eco*RI-digested Charon 16A genomic clones into the *Eco*RI site of pBR322. All procedures conformed to the NIH guidelines for recombinant DNA research. The probes V_H-81X and V_H-81Y were prepared by dissecting the indicated restriction fragments from each clone and then purifying these by agarose gel electrophoresis and labelling with ³²P by nick-translation as previously described¹⁸. Lower part, expression of rearranged V_H genes in 18-81 clones. Approximately 10 μg of poly(A)-containing RNA from 81A-20 (lane 1) and 81A-2 (lane 2) were fractionated by methylmercury hydroxide agarose gel electrophoresis, transferred to diazotized paper and assayed for hybridization to probe V_H-81Y (a) or V_H-81X (b).

to any RNA in 81A-20 cells (Fig. 4b, lane 1), consistent with the loss of this allele from the cell line (see above). This probe did, however, hybridize to a 2.7- and 2.4-kb μ_m- and μ_s-sized RNA doublet in 81A-2 RNA (Fig. 4b, lane 2) but these sequences apparently result from a non-productive rearrangement at the unexpressed allele and do not encode normal μ proteins (see ref. 20). These results confirm that the 81A-2 clone has undergone a class switch from μ to γ2b production using the same rearranged V_H-J_H region to make both types of RNA. Furthermore, in all these lines, only the RNA sequences hybridizing to the V_H-81Y probe showed a significant response to LPS treatment, suggesting that LPS sensitivity was a property of the expressed allele.

Derivatives of 18-8 accumulate deletions in the J_H-C_μ intron during growth in culture

To analyse further the deletions which occurred near the C_μ gene in the 18-8 cell lines, we prepared a ³²P-labelled probe from the major *Xba*I fragment lying within the J_H-C_μ intron (Fig. 3) and used it to analyse the size of homologous fragments in *Xba*I-digested DNA from the various lines. As expected, this probe labelled a prominent 5.5-kb *Xba*I fragment in embryonic DNA (Fig. 5, lane 1). In DNA from the 18-8 line, the intron probe labelled both the 5.5-kb fragment as well as a series of smaller fragments ranging in size to <3 kb (Fig. 5, lane 2). This suggested that the 18-8 line was accumulating deletions in this region during growth in culture. Similar analyses revealed no size heterogeneity of restriction fragments representing sequences lying 5' or 3' to those represented by the intron probe (data not shown), supporting the proposal that

sequences within the region covered by the probe have a predisposition for deletion, as suggested by others²⁴.

Additional support for the suggestion that 18-8 accumulated J_H - C_μ deletions during culture was provided by the observation that DNA from the 81A-20 and 81A-2 clones did not contain *Xba*I fragments of embryonic size that hybridized with the intron probe (Fig. 5, lanes 3 and 4). Although both C_μ alleles appeared to be present in the 81A-2 line, the *Xba*I-digested DNA from this line contained only one obvious fragment of ~2.3 kb which hybridized to the intron probe (Fig. 5, lane 3). Analyses using other enzymes and probes indicated that the lack of a second hybridizing fragment probably resulted from a deletion in the second allele which removed most of the sequence homologous to the probe (data not shown), a result consistent with the small size (9.0 kb) of one of the C_μ -containing *Eco*RI fragments in this line (Fig. 3a, lane 4). Similarly, from other experiments, the large size (5 kb) and weak hybridization of the single hybridizing *Xba*I fragment in 81A-20 DNA (Fig. 5, lane 4) seems to have resulted from a large deletion removing the first two *Xba*I sites directly 3' to the J_H segments (Fig. 3), the 5' *Xba*I site now being provided by sequence associated with the rearranged V_H gene.

Mechanism of switching

Previous evidence has suggested that cells producing μ chains can switch to production of other heavy-chain isotypes^{8,25,26}, but only in the case of cells that have activated δ synthesis has evidence been provided that the same V_H region is expressed first with μ and then with the derivative heavy chain²⁷. The present report shows that an A-MuLV transformant which has switched from μ to $\gamma 2b$ synthesis, uses for its $\gamma 2b$ protein what appears to be the same V_H region as used by the parental line for its μ protein. The apparent identity of the V_H regions used

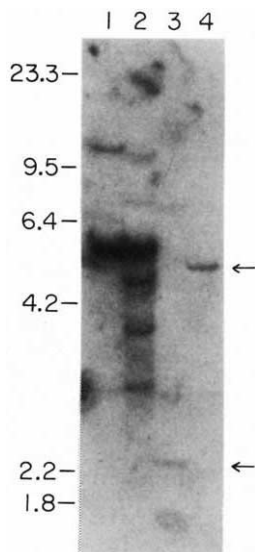


Fig. 5 Deletions within the J_H - C_μ intron. The J_H - C_μ intron probe was prepared by dissecting the 3.5-kb *Xba*I fragment corresponding to the major 5.5-kb *Xba*I fragment in the J_H - C_μ intron (see Fig. 3) from a genomic clone (pRI- C_μ) which has a 10.5-kb insert containing the C_μ gene⁴. The insert of this plasmid corresponds to the embryonic 12.5-kb *Eco*RI fragment which contains the C_μ exons (see Fig. 3) but during cloning has undergone a ~2-kb deletion several kb 5' to the first C_μ exon. The size of the excised *Xba*I fragment (3.5 kb) was a result of this deletion. Approximately 10 μ g of DNA from the indicated sources were digested with *Xba*I, fractionated by electrophoresis through 1% agarose gels, transferred to nitrocellulose paper and assayed for hybridization to the J_H - C_μ intron probe labelled with ³²P by nick-translation. DNA from: lane 1, BALB/c embryo DNA; lane 2, 18-8; lane 3, 81A-2; lane 4, 81A-20.

in the μ and $\gamma 2b$ proteins is indicated by the following: the V_H genes joined to the J_H regions on the two chromosomes in 18-8 cells and its derivatives have been molecularly cloned and are denoted V_H -81X and V_H -81Y; a μ -producing 18-8 derivative has lost one allele but retained V_H -81Y in its μ mRNA, identifying this as the V_H that encodes the μ protein; the $\gamma 2b$ mRNA in the $\gamma 2b$ -producing derivative hybridized to V_H -81Y but not V_H -81X, identifying V_H -81Y as the V_H region that encodes the $\gamma 2b$ protein and showing that the μ and $\gamma 2b$ proteins share V_H regions. Actually, the identity of V_H regions on the two mRNAs was established mainly by cross-hybridization, but based on the lineage relationships of these lines, the possibility that the cross-hybridizing V_H regions are not identical is very low.

The mechanism of heavy-chain switching has previously been inferred from observations on the configuration of the heavy-chain genes in myeloma cells, tumour cells that represent terminally differentiated cells of the B-lymphoid lineage. These studies have indicated that class switching occurs by a deletional mechanism⁹⁻¹⁶, and this process is apparently mediated by switch recombination sites, a set of which lies 5' to each of the C_H genes^{15,16,28,29}. Thus we expected that the switch of 18-81 cells from μ synthesis to $\gamma 2b$ synthesis would be accompanied by a deletion of at least one copy of the C_μ gene segment in these cells. However, the γ -producing 81A-2 line described here retains two copies of the C_μ gene, each of which still appears to be associated with the V_H complex (Fig. 3). Because digests using other enzymes suggest the possibility of an alteration within or near the C_μ gene on one of the alleles (F.W.A. and D.B., unpublished results), additional molecular cloning analyses will be necessary to confirm the linkage. However, analysis of the $\gamma 2b$ gene in these lines using a series of different restriction endonucleases revealed no obvious alterations near either the $\gamma 2b$ gene or its flanking sequences (data not shown). In addition, there are examples of at least two other A-MuLV-transformed lines that apparently have undergone a switch to γ production without loss of a C_μ gene (see ref. 20). The data available imply that the switch to $\gamma 2b$ production in A-MuLV transformants results from transcription through the C_μ gene, with deletion of the C_μ and other intervening sequences occurring by a post-transcriptional processing mechanism. Such a mechanism would be analogous to that proposed as mediating the transition from μ_m to μ_s RNA production^{18,30,31} and the simultaneous expression of μ and δ genes by the same cell^{27,32,33}, and predicts either a very long (>50 kb) primary transcript or a rearrangement placing the $\gamma 2b$ gene closer to μ , perhaps in the place of δ . Whatever the mechanism, this line should provide an excellent model system for studying various aspects of the class-switching process.

It has been suggested that the occurrence of class switching in 18-81 cells demonstrates that pre-B lymphocytes can carry out the class switch event⁷. Although it is true that most A-MuLV-transformed cells appear closely related to pre-B lymphocytes, 18-81 may be an exception; as opposed to most other A-MuLV transformants, it gives rise to subclones having rearranged κ light-chain genes^{4,5} some of which produce κ protein^{6,34}, it makes predominantly the secreted form of μ mRNA (Fig. 1), and it probably produces a low amount of J chain (M. Koshland, personal communication). These characteristics suggest that 18-81 is a cell line having a propensity to mature beyond the pre-B-lymphoid cell stage. Thus, the relatively high frequency of γ -producing cells generated by this line may be a consequence of its maturational potential as opposed to an event of the pre-B lymphocyte.

Class switching in cultured cells

Other laboratories have been able to detect among cultured myeloma and hybridoma cells, variants that express a new heavy-chain isotype³⁵⁻³⁷. In these experiments, powerful mutagenic and selective methods were used to recover the very rare class-switch variants which appear in non-mutagenized

cells at a frequency of $<10^{-6}$ per cell per generation^{36,37}. Our analyses, together with those of Burrows *et al.*⁷, suggest that μ to $\gamma 2b$ class switching is continually occurring in the 18-8 cell line and in some isolates may occur at a frequency as high as 10^{-2} per cell per generation.

A second difference between the selected myeloma and hybridoma variants and A-MuLV transformants is that the 81A-2 line has switched from μ to $\gamma 2b$ expression, skipping the intervening $\gamma 3$ and $\gamma 1$ C_H regions, whereas the selected variants generally switch to a neighbouring C_H gene³⁵⁻³⁷. The switch from μ to a different heavy-chain isotype is thought to occur normally during B-cell differentiation. Switching from one γ subclass to a different subclass—as occurs in the myeloma variants—has not been reported for normal B cells and the significance of this type of low-frequency switching in permanent cell lines is not clear. A μ to δ class-switch variant has been described³⁷ and probably results from deletion of the C_μ gene²⁷.

Regulation of μ expression

The high level of μ -specific protein and RNA expressed by the 18-8 and 18-81 A-MuLV-transformed cell lines (0.1% of soluble protein; see ref. 2) is relatively unique among μ -positive, light chain-negative A-MuLV-transformants, which generally produce ~ 10 -fold lower levels. Previous studies have demonstrated that the production of high levels of heavy chains has an adverse effect on the growth of plasmacytoma lines that have lost light-chain gene expression³⁸. In view of this result, the decline of μ production in long-term cultures of the μ -only 18-8 lines most probably results from the selective outgrowth of cells in which μ production has decreased, while the relative stability of μ levels in many μ -only A-MuLV-transformed lines (N.R. and F.W.A., unpublished results) may reflect the low levels of the protein produced initially.

The decreased μ production in various 18-81 isolates is usually inducible to the original high levels on treatment of the cultures with LPS. We have as yet little information on the genetic basis of the decreased expression or induction phenomena. However, note that in the 81A-2 clone that has switched to $\gamma 2b$ production, $\gamma 2b$ protein and RNA sequences show an extent of LPS induction similar to that observed for μ sequences in the parental line whereas the defective μ mRNA sequences produced from the second allele show little or no induction (Fig. 1). This result suggests that the structure mediating the induction is associated with the expressed V_H gene or its flanking sequences and is presumably an element of DNA structure. In this regard it is significant that in the two low-level μ producers that are LPS-inducible, only a single copy of the μ gene was apparent, and this had undergone an extensive deletion in the intron between J_H and C_μ . An intriguing possibility is that a sequence in the deleted region has a positive involvement with μ production and that loss of that sequence can be overcome by LPS treatment. It should be noted, however, that deletions within this region of up to 2.8 kb are compatible with high-level μ production in myelomas³⁹.

The deletions that occur within the J_H - C_μ intron during propagation of the 18-8 line may be related to strain-associated length variations in that region²⁴ as well as to deletions within that region which are observed in myelomas and which occur

when C_μ genes are cloned and propagated in *Escherichia coli*^{15,16,24,28}. The latter two phenomena have been ascribed to a propensity of the S_μ region—which lies ~ 2 kb 5' to the C_μ gene—to promote deletions^{15,16,24,28}. Although many of the deletions within this region observed in the 18-8 populations may be analogous in size to those described for myelomas or molecular clones (~ 2 kb), the deletions observed in some of the 81A clones are much larger (>5 kb). However, these deletions do appear to have their 3' termini near the S_μ region. Whether or not all these deletions are also mediated by the S_μ region remains to be determined. However, an intriguing possibility is that the high rate of deletion within this region is related to the propensity of the 18-8 line to undergo class switching. Most A-MuLV-transformed lines have deletions within this region (not shown) and the only other line examined in detail, 300-18, also accumulated these deletions during the growth in culture (F.W.A. and D.B., unpublished observations). However, of five permanent T-cell lines examined, all of which had been in long-term culture, none showed evidence of deletions within this region, suggesting that the activity responsible may be B-cell specific.

Conclusion

These data emphasize the variety of phenotypes to be found among the many cell lines transformed by A-MuLV. In this case, subclones from a single transformant, 18-81, have shown a switch from μ to $\gamma 2b$ synthesis and a progressive loss of heavy-chain gene expression. Analysis of both events has uncovered aspects that may be important to the general understanding of immunodifferentiation.

The switch from synthesis of μ to synthesis of $\gamma 2b$ was accomplished without the expected loss of a C_μ gene, which suggests that class switching beyond the δ gene may be, at least partly, a transcriptionally controlled event in contrast to previous evidence for a deletional mechanism⁹⁻¹⁶. Deletion certainly occurs, but it may be secondary to a transcriptional (or RNA processing) switch. The progressive loss of heavy-chain synthesis has two interesting aspects: it can be reversed by LPS treatment of cells and can be correlated with deletion in the J_H - C_μ intron. Although the causative relationships have not been proved, the results suggest that transcription of heavy-chain genes (both μ and $\gamma 2b$) or the processing of initial transcripts may be regulated by DNA sequences in the J_H - C_μ intron. Both the amount of mRNA produced and the control of mRNA levels by LPS were correlated to the deletion; if these measurements reflect transcriptional activity, immunoglobulin gene expression may be regulated by DNA elements in the J_H - C_μ intron rather than any upstream of the $V_H J_H C_H$ complex. This is a reasonable assumption because otherwise each V_H gene would be required to carry a complete regulatory region 5' to its structural gene. The way in which elements of the intron may regulate transcription requires further study.

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LETTERS TO NATURE

Speckle observations of the nucleus of NGC1068

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The nuclei of Seyfert galaxies contain very turbulent motion and are often intense sources of radio emission. It has been proposed that very compact supermassive objects could be the source of these energetic phenomena. Speckle observations of the nucleus of NGC1068 have revealed for the first time the presence of such an object with a diameter of ≤ 2.3 pc but emitting an amount of visible light equivalent to that from 5×10^9 solar masses.

NGC1068 is an Sb spiral galaxy¹ with a Seyfert nucleus² which is at distance³ of 16 Mpc (assuming $H_0 = 75 \text{ km s}^{-1} \text{ Mpc}^{-1}$). Several observers^{3–6} have studied the broad ($\approx 1,450 \text{ km s}^{-1}$) [O III] emission lines from the central (≈ 300 pc diameter) regions. There are at least four distinct clouds with separate velocities within this range and the total kinetic energy³ in this turbulent ionized gas is $\approx 9 \times 10^{54}$ erg. Bertola⁶ has detected a stellar-like inner nucleus with a diameter of ≤ 160 pc (≈ 2 arc s) which emits 9% ($\approx 12 M_\odot$) of the total visible continuum of the galaxy. The variability⁷ of this optical emission has suggested that this may originate within a volume as small as a few light months.

High-resolution (≈ 1 arc s) radio observations of the nuclear regions of NGC1068 by the Jodrell Bank MERLIN array⁸ and VLA⁹ reveal that an intense source of non-thermal radio emission of ≈ 77 pc diameter (≈ 1 arc s) is coincident with this central object. This is of size comparable with the upper limit of the $10 \mu\text{m}$ IR structure¹⁰, whereas radio VLBI measurements^{11,12} have, so far, failed to detect emission on an angular scale of ≥ 0.1 arc s.

Speckle observations of the optical continuum emission from the central active nucleus of NGC1068 permit its angular structure from 0.03 to 0.9 arc s (2.3 to 70 pc) to be investigated. The nucleus of NGC1068 was observed with the Imperial

College of Science and Technology (London) speckle interferometer^{13–17} combined with the $f/15$ focus of the 3.9 m Anglo-Australian Telescope.

This device has an EMI, four-stage, image tube (S20) combined with a Plumbicon TV camera and detects individual photon events, (with the combination of lenses used for NGC1068) over the image of a 3.07 arc s^2 square ($\approx 256 \times 256$ pixels) field. An individual recording is obtained, with an exposure time of $1/50$ s, of this atmospherically distorted image which is then auto-correlated on-line within $1/25$ s. Over an extended observing period these separate, two-dimensional auto-correlations are co-added and finally an auto-correlation is obtained for the 3.07 arc s^2 area in which objects separated by ≤ 0.9 arc s and ≥ 0.03 arc s (the limiting angular resolution of the telescope at $5,000 \text{ \AA}$ for instance) are capable of being detected. This is stored on tape in an array from -64 to $+63$ pixels in the x dimension (right ascension) and from 0 to -63 pixels in the $-y$ dimension (declination) where the 00 pixel is the zero of the two-dimensional auto-correlation and is the centre of the narrow 'photon spike'.

The integrated auto-correlation of the field, which contains the object under investigation, is then compared with that for a star, known to be single, near in angular distance to the object and of nearly equal brightness (a neutral density filter can be used). If the observations of this reference star are made immediately after those on the object, for the same integration time and through the same filter then it can reasonably be assumed that the statistical properties of the turbulence in the atmosphere are similar in both cases and a significant comparison of the auto-correlations can be made.

The observations of the nucleus of NGC 1086 with this system are summarized in Table 1. (The $5,007 \text{ \AA}$ emission line was shown to be negligibly faint in a separate integration through a narrow filter.) Figure 1 shows a grey-scale representation (using the Manchester node of the STARLINK network) of the two-dimensional auto-correlation obtained for NGC1068 and emphasizes the very faint features (D–E) which are produced by separate sources within the 3.07 arc s^2 area that is auto-correlated but which are ≥ 200 times fainter than the single dominant source. This is primarily responsible for the intense and narrow central feature (around the 00 pixel), which is 'burnt out' in Fig. 1. However, in Fig. 2 the cut in the y direction through this central feature, from the 00 pixel, is compared with the same cut through the auto-correlation for the reference star (see Table 1). This was scaled to give the same number of counts in the photon spike around (the 00 pixel) as that obtained for NCC1068.

Table 1 Speckle observations of NGC1068 and reference star

Object	Field size (arc s)	Field centre (1950)	Filter ($\text{\AA}$)	Integration time (s)	No. of separate frames autocorrelated
Nucleus NGC1068	3.07×3.07	$\alpha = 2 \text{ h } 40 \text{ m in } 07.1 \text{ s}$ $\delta = 00^\circ 13' 31.4''$	5,000–5,600	1,160	2.89×10^4
Ref. Star ($M_v = 9.0$)	3.07×3.07	$\alpha = 2 \text{ h } 41 \text{ m in } 11.33 \text{ s}$ $\delta = 00^\circ 0' 2.3''$	5,000–5,600 (ND = 1.0)	850	2.125×10^4

Table 2 Sources in the auto-correlation of NGC1068

Source	M_v	Separation from primary (arc s)	Separation from primary (pc)
A	11.8 ± 0.3	0	0
B	17.7 ± 0.05	0.51 ± 0.03	40
C	19.0 ± 0.05	0.27 ± 0.03	21
D	18.2 ± 0.05	0.47 ± 0.03	37
E	17.4 ± 0.05	0.60 ± 0.03	47

The auto-correlation displayed in Fig. 1, and the cut through this and the reference star in Fig. 2, demonstrate that the nucleus of NGC1068 is dominated by a stellar-like object (source A) which is unresolved by the 0.03 arc s resolution of this system (this is a conservative upper limit to this diameter). A comparison of the counts in this central region of the auto-correlation with those obtained for the reference star give a value for source A of $M_v \approx 11.8$, which is very similar to the magnitude of the continuum emission from the entire central ≈ 160 pc as estimated from the work of Burbidge *et al.*³ and Bertola⁶. This suggests that most (see Table 2 and Fig. 1) of this nuclear emission originates within a region of ≈ 0.03 arc s diameter (≈ 2.3 pc) with a luminosity of $\approx 5 \times 10^9 L_\odot$.

Several possibilities for the nature of this dominant compact source should be considered. For example, the optical emission could be intense synchrotron radiation, though the failure to detect this object using radio VLBI techniques (angular resolution of ≈ 0.1 arc s) could be explained by synchrotron self-absorption with a turnover frequency of $\approx 10^{10}$ Hz (this implies an angular size of $\approx 10^{-4}$ arc s). Also thermal free-free absorption could be occurring in front of the source and an emission measure of only 10^8 – 10^9 pc cm $^{-6}$ would be required to make a central synchrotron radio source not observable. However, the discovery of optical circular polarization¹⁸ argues against the synchrotron hypothesis.

Alternatively, this central source could be an extremely compact star cluster with a total mass of $\approx 5 \times 10^9 M_\odot$ and a mass density of $\approx 8 \times 10^8 M_\odot$ pc $^{-3}$ if a simplistic mass-to-luminosity relationship is applied. If this is dynamically bound then a velocity dispersion of $\approx 5 \times 10^3$ km s $^{-1}$ would be required which is in excess of the observed widths of the stellar absorption lines. Also, variability⁷ of the optical continuum cannot occur in the integrated light from such a large number of separate stars.

A more likely possibility is that the compact dominant source A is a supermassive, single, condensed object comparable to that inferred to be at the centre of M87¹⁹. The existence of radio jets in Seyfert nuclei^{8,20} has been interpreted on the basis of beams emitted along the rotation axis of such objects.

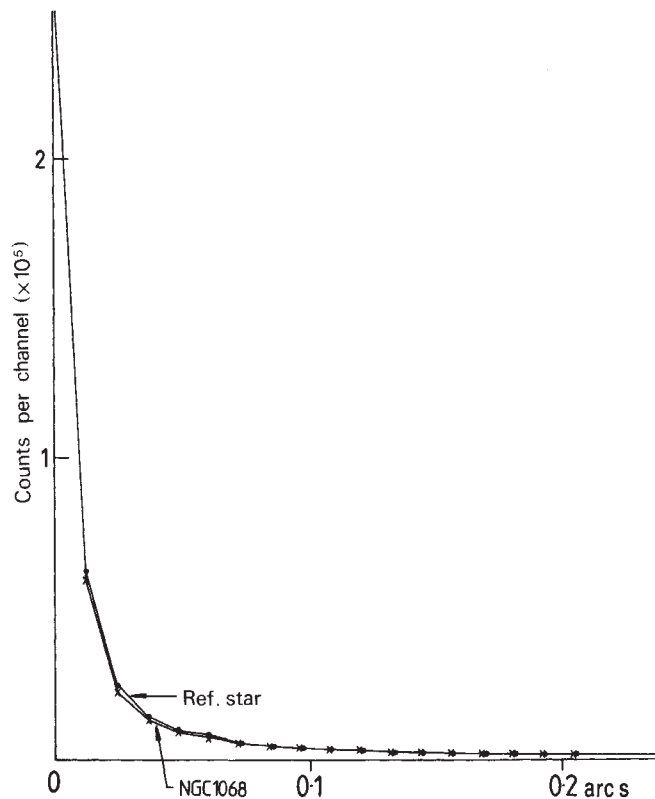


Fig. 2 The cut in the $-y$ direction through the 00 pixel for NGC1068 in Fig. 1 is compared with the corresponding cut through the auto-correlation of the point reference star.

The secondary maxima B–E in Fig. 1 could be produced by four much fainter sources with the magnitudes and separations from the primary that are listed in Table 2. Similar features do not appear on the auto-correlation of the reference star and they are thought most probably to originate within the nucleus of NGC1068 and not be caused by faint foreground stars in our Galaxy.

However, caution is required in the interpretation of the configuration in Fig. 1 for cross-correlations between these secondary sources could also appear as secondary maxima and until some detailed modelling is carried out (which will also consider orientations of the secondary sources) it can only be stated that certainly two but possibly up to four secondary, unresolved, sources are clustered around the primary compact object in the nucleus of NGC1068. Even their nature could be unusual and require similar, but less dramatic, explanations to those for the primary source.

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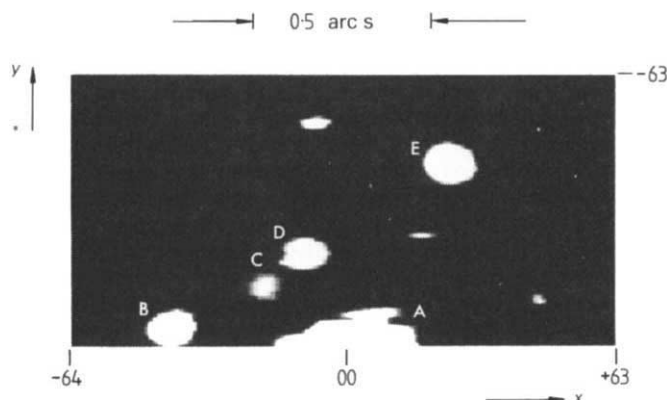


Fig. 1 A grey-scale representation of the auto-correlation of the nucleus of NGC1068. The central source A around the 00 photon spike is 'burnt out' here to permit the fainter (≈ 200 times) secondary sources B–E to be revealed. Other minor features are thought to be noise or cross-correlations. The irregularities around the primary A are also on the noise level and not thought to be real.

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A CCD image of the galactic centre

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Because the galactic centre lies behind approximately 27 magnitudes of visual extinction, observations at visible wavelengths are impossible. The recent introduction of charge-coupled devices (CCDs) to astronomy represents the first opportunity to use sensitive area detectors in the near IR. Using a cooled CCD, we have obtained an image of the galactic centre at an effective wavelength of $0.9\ \mu\text{m}$. Two unresolved sources were found, separated by 3 arc s along the galactic plane. The detection of the two sources was confirmed by a further observation the following night. The possibility that the new sources are foreground objects cannot be completely ruled out; however, their positions are remarkably close to those of the non-thermal radio source and the $2.2\ \mu\text{m}$ source IRS-16. While it is possible that the new sources could be a pair of highly reddened individual stars or compact clusters, it seems more likely that they are two compact H II regions seen in line emission.

The CCD image (Fig. 1) was taken at the prime focus of the Anglo-Australian Telescope (AAT) on 8 September 1981 during conditions of sub-arcsecond seeing. The CCD camera was built for the Anglo-Australian Observatory (AAO) by the Royal Greenwich Observatory, and uses as a detector an RCA SID 53612 chip with 320×512 pixels, giving an image scale of 0.50 arc s per pixel. The exposure time was 10 min through an RG830 (8,300 Å long-pass) filter. Although the far-red response of the CCD is not well known, the quantum efficiency is believed to fall linearly from about 60% at $\sim 8,000\ \text{\AA}$ to zero at $10,500\ \text{\AA}$, thus defining with the RG830 filter an effective bandpass of $\sim 1,000\ \text{\AA}$ centred on $9,000\ \text{\AA}$. The image has been flat-fielded by division by an exposure of blank sky—this process is necessary to remove interference fringes which are generated in the detector chip by strong night-sky emission lines.

Comparison of Fig. 1 with a IV N Schmidt plate (courtesy of the UK Schmidt Telescope Unit) allows all the stellar images to be identified with previously known stars except for the two images marked CCD1 and CCD2. The two new sources must therefore be highly reddened as would be expected for sources in the galactic plane at the distance of the galactic centre.

In the absence of spectroscopic data one cannot completely rule out the possibility that the new sources are foreground objects which appear in line with the galactic centre purely by chance. This would require a remarkable coincidence, however, as their positions are very close to those of the non-thermal radio source and the $2.2\ \mu\text{m}$ source IRS-16, which are normally assumed to mark the galactic centre. No other very red objects are prominent on the CCD frame. In addition, Allen, Carter and Malin (personal communication) have made a partial map with 2 arc s resolution of the immediate vicinity of these objects in Brackett γ and find two corresponding peaks in the line intensity. Although Brackett γ emission is present in Be stars, the probability of finding two such stars of almost identical magnitude within 3 arc s of a given point is infinitesimal.

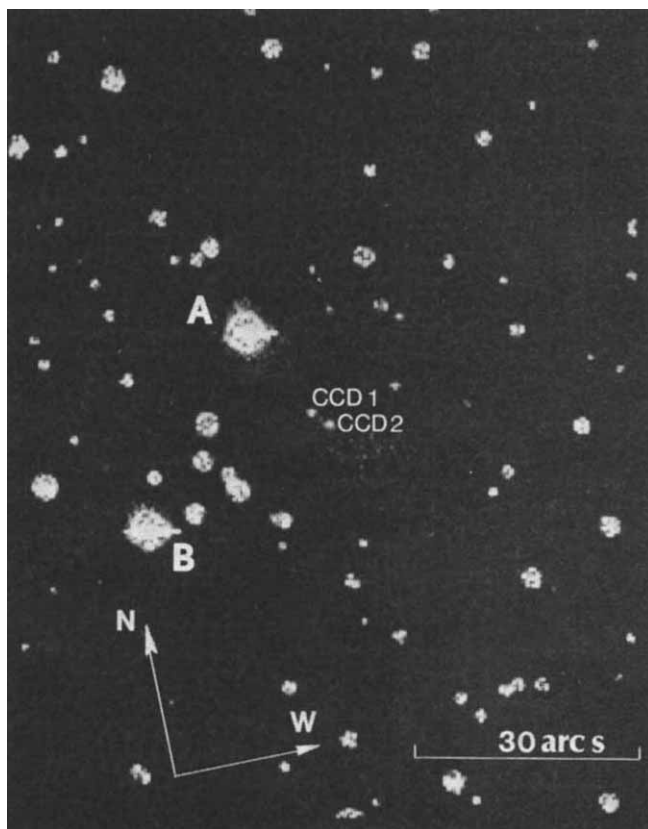


Fig. 1 CCD photograph of the galactic centre at an effective wavelength of $0.9\ \mu\text{m}$. All the stellar images are also seen on a IV N plate, with the exception of those marked CCD1 and CCD2. The bright stars identified as A and B are field stars used for positional information.

Positions for the new sources were obtained by measurement relative to two brighter visible field stars, shown as A and B on Fig. 1. These stars were in turn identified on the Palomar Sky Survey E plate and their positions determined relative to 15 nearby Smithsonian Astrophysical Observatory stars by measurement of the plate on a microdensitometer, followed by a six-parameter least-squares fit. The positions of all four stars are listed in Table 1, and should be accurate to better than 1 arc s. Star A is the visible star identified on the $2.2\text{-}\mu\text{m}$ map of Becklin and Neugebauer¹; the position they determined agrees with the present value to within 0.8 arc s in each coordinate.

The intensities of the new galactic centre sources were determined by calibration against the white dwarf LDS749B, whose magnitude at $9,000\ \text{\AA}$ can be derived from the work of Oke². The resulting values are 18.9 ± 0.5 for CCD1 and 19.1 ± 0.5 for CCD2. This is equivalent to an observed flux of $1.9 \times 10^{-21}\ \text{W cm}^{-2}$ from each source, corresponding to a dereddened flux of $2.2 \times 10^{-16}\ \text{W cm}^{-2}$ for a visible extinction of $A_V = 27\ \text{mag}$ (ref. 3) and assuming the extinction follows Van de Hulst's curve number 15 (ref. 4).

The galactic centre has previously been detected at 1.06 and $1.18\ \mu\text{m}$ using a 22 arc s aperture⁵. The observed magnitudes were 13.9 and 12.4 respectively. Comparison with the present result suggests that most of the flux seen by Spinrad *et al.*⁵ must have come from extended emission. There is some indication of nebulousity on the CCD frame, but its reality requires confirmation.

The shortest IR wavelength map available is that by Becklin and Neugebauer¹ at $2.2\ \mu\text{m}$. In this map, the central source (IRS-16) is seen to be slightly extended. The resolution used (2.5 arc s) is not sufficient to indicate whether the images seen by the CCD are also visible at $2.2\ \mu\text{m}$; however, they clearly are not prominent. In an early radio work at high resolution

Table 1 Positions of galactic centre sources

Object	RA (1950.0)	Dec (1950.0)
A	17 h 42 min 30.06 s	-28° 59' 01.2"
B	17 42 31.63	-28 59 27.3
CCD1	17 42 29.53	-28 59 15.0
CCD2	17 42 29.47	-28 59 17.4
IRS-16*	17 42 29.3	-28 59 18
Radio source†	17 42 29.335	-28 59 18.60

* Position from ref. 1.

† Position from ref. 7.

but with limited $u-v$ plane coverage, Balick and Brown⁶ proposed a model in which the non-thermal radio source consists of two components separated by 2.4 arc s and oriented almost north-south. Although this model has some similarity with the CCD data, further observations by Balick and Brown led them to reject it in favour of a single-component model. A more recent VLA map⁷ makes it clear that the powerful non-thermal radio source at the galactic centre does not have a double structure on the scale of arcseconds. This map (at 5 GHz) is, however, limited to a resolution of 2×8 arc s, which is insufficient to allow proper identification of the CCD images. Unfortunately, the different astrometric reference frames used at radio and optical wavelengths do not allow registration of the two maps to better than ~ 1 arc s. Thus one cannot rule out completely the possibility that the non-thermal radio source lies between the two newly-discovered objects, although this seems unlikely. The IR map can, of course, be precisely placed with respect to the CCD image by means of the visible field stars.

If the powerful 2.2- μ m source IRS-7 is a late M supergiant, as is normally assumed, its apparent magnitude at 0.9 μ m would be around 20. This is not much fainter than the sources detected by the CCD, and suggests that a deeper exposure would also detect this source.

If the flux from each of the newly discovered objects is due to stellar continuum at 10 kpc, then each source has a de-reddened absolute magnitude in I of about -7.7 . Because each image is unresolved it would be reasonable to ascribe all of this flux to a pair of individual stars. Each star would then need to be a supergiant, of spectral type K or earlier. This interpretation would be consistent with the objects being unimpressive at 2.2 μ m, and with the lack of pronounced CO absorption⁸.

The present results represent the shortest IR wavelength at which the galactic centre has been observed. It is therefore tempting to try to identify these objects with the ionization source for the region. Although the total flux required to power the 10 μ m H II regions could be provided by a single O5 star, Lacy *et al.*⁹ have shown that this flux must be unusually soft, that is $T_{\text{eff}} \approx 35,000$ K (see also ref. 10), corresponding to something closer to an O9 star. It thus seems unlikely that a single pair of stars could be responsible for all the ionization. One cannot, however, rule out the possibility that the stars seen by the CCD are members of a larger cluster, whose overall ionizing output powers the region.

An alternative interpretation would be that the CCD images are due to line emission from two compact H II regions. The most plausible lines are the [S III] $^1D \rightarrow ^3P$, $J = 2 \rightarrow 1$ and $J = 2 \rightarrow 2$ transitions at 9,069 and 9,532 Å respectively, with some additional contribution from the Paschen series of hydrogen. The He I 10,830 Å line would probably be too far down on the CCD response to be detectable. Assuming that all of the Brackett γ flux seen by Bally *et al.*¹¹ in an 8 arc s beam is due to the two compact sources, it is straightforward to calculate the expected [S III] flux under the assumption that the ionization state is similar to that of typical galactic H II regions. The resulting figure of 2×10^{-16} W cm⁻² is consistent with the observed, de-reddened value. Because of the very high spatial resolution of the CCD frame, it is not surprising that the two newly discovered objects are not prominent on previous, lower-resolution maps of the radio and Ne II emission.

The similarity of the two CCD images suggests that if the objects are compact H II regions, they may be ionized by a common source midway between them. Lacy *et al.*¹² have discussed in detail the possible existence of a $10^6 M_{\odot}$ black hole at the galactic centre. The ionizing flux from such an object would be more than sufficient to power the two compact sources found. We note that the separation of the sources (4×10^{17} cm) is 5,000 times greater than the expected diameter of the optically thick accretion disk of such a black hole.

The present data do not allow a unique identification of the sources detected by the CCD. It is of prime importance to obtain complete high spatial resolution maps of the region both in the 2.2 μ m continuum and in Brackett γ , and to attempt to obtain spectra of the sources at 9,000 Å. This work will be attempted at the AAT as soon as possible. Meanwhile, radio maps at even higher resolution than the present VLA data would be desirable, as would improved registration of the optical and radio frames of reference. If the galactic centre sources turn out to be emitting strongly in [S III], then observations of the [S IV] line at 10.5 μ m would yield a precise determination of the temperature of the ionizing radiation field. This would, in turn, place tight constraints on the nature of the ionizing source.

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A simple ice sheet model yields realistic 100 kyr glacial cycles

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Records of global ice volume for the past 700 kyr, based on oxygen isotopic data from deep-sea cores and reflecting mainly the changing Northern Hemispheric ice sheets, show a dominant cycle of roughly 100 kyr period. The records also show smaller-amplitude oscillations with spectral peaks at roughly 40 and 20 kyr periods, which are well correlated with the Milankovich insolation variations due to perturbations in the Earth's orbital parameters. However, no model has accurately simulated the 100 kyr glacial cycle. Recently Birchfield *et al.*¹ and Oerlemans² have obtained encouraging agreement with some features of the glacial cycle by using a simple ice sheet model with a realistic time lag in the response of the bedrock to the ice load. This study extends their basic model, first by including topography to represent high ground in the north. Improved results can then be obtained but only with unrealistic parameter values and for some aspects of the record. Further improvements are

obtained by crudely parameterizing possible calving at the equatorward ice sheet tip during deglaciation by proglacial lakes and/or marine incursions from the Atlantic, as emphasized by Andrews³. The resulting ice volume curves agree fairly well with the observed records and their power spectra over the past 700 kyr.

The ice sheet model^{1,2} predicts ice thickness in a cross-section running approximately north-south along a typical flow line (see Fig. 4). A vertically integrated approximate ice flow law is used with east-west flow neglected, which reduces the ice dynamics to a non-linear diffusion equation for ice thickness h

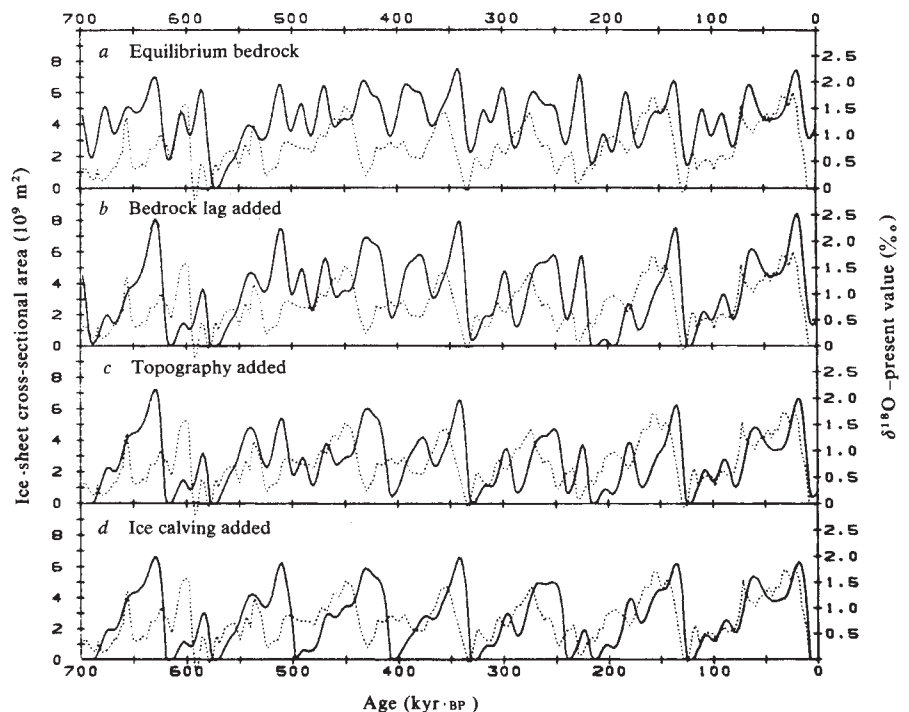
$$\frac{\partial h}{\partial t} = A \frac{\partial}{\partial x} \left[h^\alpha \left| \frac{\partial(h+h')}{\partial x} \right|^\beta \frac{\partial(h+h')}{\partial x} \right] + G(h+h', x, \text{orbit}) \quad (1)$$

where t is time and x is distance to the south. h' is the elevation of the bedrock surface above a fixed reference level (taken as the present mean sea level). For all results below $A = 5.77 \times 10^{-4} \text{ m}^{-3} \text{ yr}^{-1}$, $\alpha = 5$ and $\beta = 2$. The northern boundary of the model is taken at 74°N to represent the Arctic Ocean shoreline, where h is required to be zero (this excludes the possibility of marine ice sheets forming in the Arctic⁴). G is the net annual mass balance on the ice surface and crudely represents the current distributions of snowfall and ice melt. Its dependence on the surface elevation $h+h'$ follows Oerlemans²:

$$G = \begin{cases} a(h+h'-E) - b(h+h'-E)^2 & \text{if } h+h'-E \leq 1,500 \text{ m} \\ 0.56 & \text{if } h+h'-E > 1,500 \text{ m} \end{cases} \text{ m yr}^{-1} \quad (2)$$

where $a = 0.81 \times 10^{-3} \text{ yr}^{-1}$, $b = 0.30 \times 10^{-6} \text{ m}^{-1} \text{ yr}^{-1}$. At elevations below the equilibrium-line altitude E , G becomes rapidly negative (due mainly to summer air temperatures being above freezing), whereas above E , G increases more slowly to a maximum value. As in previous studies, E is assumed to have a constant slope in latitude and to be shifted uniformly by the insolation variations according to $E = E_0(x) + k\Delta Q$, where E_0 is the present equilibrium line and ΔQ is the difference in the summer half-year insolation at 55°N from that of the present, calculated from the orbital parameters⁵. E_0 and the insolation sensitivity, k , are specified for each run below.

Fig. 1 Total cross-sectional area of ice versus time for various model versions (solid curves). The corresponding approximate ice volume can be obtained by multiplying by a typical east-west ice sheet dimension ($\sim 3,000 \text{ km}$). The dotted curve in each panel is an oxygen isotope deep-sea core record minus its present value, redrawn from ref. 20. *a*, Model run with no topography ($h'_0(x) = 0$) and with the bedrock in isostatic equilibrium ($\nu = \infty$, $h' = -rh$); the present equilibrium line $E_0(x)$ passes through the (lat. ($^\circ \text{N}$), height (m)) point (73, 0) with a slope of 0.9×10^{-3} ; the insolation sensitivity $k = 25 \text{ m ly}^{-1} \text{ day}$; the ice/rock density ratio $r = 0.3$. *b*, As *a* except with bedrock lag $\nu = 100 \text{ km}^2 \text{ yr}^{-1}$ (in this and subsequent model runs, the maximum ice areas attained correspond to southern tip positions of ~ 49 – 52°N). *c*, Model run with piecewise-linear topography $h'_0(x)$ joining the following (lat., height) points: (72, 0), (70, 850), (66, 200), (40, 400), (30, 400); the northern model boundary is at 72°N ; $E_0(x)$ passes through (66, 850) with a slope of 1.0×10^{-3} ; $k = 20 \text{ m ly}^{-1} \text{ day}$; $\nu = 100 \text{ km}^2 \text{ yr}^{-1}$; $r = 0.4$. *d*, Model run including the calving mechanism (equation (4)); $h'_0(x)$ joins (74, -500), (70, 850), (66, 200), (40, 400), (30, 400) (the calving mechanism operates permanently at the northern tip, maintaining it at $\sim 72.5^\circ \text{N}$); $E_0(x)$ passes through (70, 550) with a slope of 1.0×10^{-3} ; $k = 17 \text{ m ly}^{-1} \text{ day}$; $\nu = 100 \text{ km}^2 \text{ yr}^{-1}$; $r = 0.3$; sea level $S = 0$ in equation (4).



The response of the bedrock to the changing ice load consists of an elastic deformation of the lithosphere and a deeper viscous flow in the asthenosphere. As in other models^{1,2} the smaller elastic deformation is neglected here. It is not generally agreed whether the best asthenospheric model is closer to a relatively thin channel or a deep half-space⁶⁻⁸; the thin-channel model is used here, which with linear viscosity yields^{6,9}

$$\frac{\partial h'}{\partial t} = \nu \frac{\partial^2}{\partial x^2} [h' - h'_0(x) + rh] \quad (3)$$

where r is the ratio of ice density ρ_i to rock density ρ_a ; ν is $\rho_a g H^3 / N \eta$ where g is the gravitational acceleration, H is the mean thickness of the channel, η its dynamic viscosity, and N is 12 or 3 (depending on whether zero tangential velocity or zero tangential stress is required at the base of the lithosphere). $h'_0(x)$ is the surface topography due to crustal structure that would prevail in isostatic equilibrium without any ice. The choice of lateral boundary conditions for equation (3) is uncertain; for the runs below it was required that $h' = h'_0$ at the model boundaries 74°N and 30°N , but other reasonable choices have little effect on the results. The main effect of using equation (3) instead of a local-response equation^{1,2} is that the bedrock adjusts rapidly to small-scale ice loading, whereas the broad deep depressions under the largest ice sheets can be preserved for a much longer time during subsequent deglaciations.

Equations (1) and (3) were numerically integrated forward in time using a Newton-Raphson scheme for each equation individually, with an implicit contribution from every term involving h in the ice flow expression in equation (1). This allowed a time step of 50–100 yr with a latitudinal spacing of 55.5 km ($= 0.5^\circ \text{ lat.}$). Tests were made to check that the results agree adequately with those at higher resolutions. Many runs were made varying the parameters E_0 , k , r , ν and $h'_0(x)$, and the results are summarized in Fig. 1. For comparison a deep-sea core record is shown in each panel (dotted curve); this is interpreted here as being proportional to Northern Hemispheric ice volume, although it may also have been affected significantly by Antarctic ice sheet variations¹⁰.

Figure 1a shows a run with no topography ($h'_0(x) = 0$) and with the bedrock always in isostatic equilibrium with the ice load ($\nu = \infty$, that is $h' = -rh$). This is similar to results of earlier

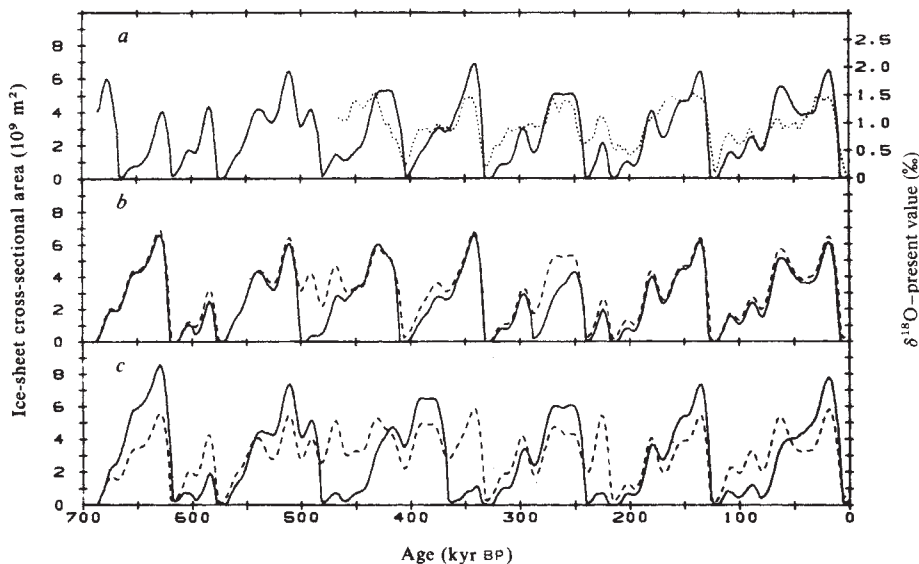


Fig. 2 *a*, Solid curve, as Fig. 1*d* except sea level $S = -100 \times (\text{ice cross-sectional area}/6 \times 10^9 \text{ m}^2) \text{ m}$, and with different initial conditions. Dotted curve, oxygen-isotopic deep-sea core record minus its present value, from the cores RC11-120 and E49-18, redrawn from ref. 29. *b*, Solid curve, as Fig. 1*d* except sea level $S = 100 \text{ m}$. Dashed curve, as Fig. 1*d* except sea level $S = -100 \text{ m}$. *c*, Solid curve, as Fig. 1*d* except $\nu = 30 \text{ km}^2 \text{ yr}^{-1}$. Dashed curve, as Fig. 1*d* except $\nu = 300 \text{ km}^2 \text{ yr}^{-1}$.

plastic ice sheet models^{11,12}, adequately reproducing the observed 20 and 40 kyr oscillations but with no trace of a 100 kyr cycle. The main features that this model lacks are the rapid retreats from glacial to interglacial conditions, for instance the last deglaciation from ~ 18 to ~ 6 kyr BP. These retreats are responsible for the asymmetric 'sawtooth' shape of the 100 kyr cycle and may even be essential to its existence. If the magnitude of the orbital forcing is increased in this model, the amplitude of the oscillations simply increases and also the ice sheet can vanish for extended periods, as in the earlier plastic models; however, no real resemblance to the observed glacial cycle can be obtained.

Figure 1*b* is produced with no topography but with $\nu = 100 \text{ km}^2 \text{ yr}^{-1}$, corresponding to a bedrock lag time of ~ 10 kyr for the broadest features⁶. This curve is similar to that in ref. 1, with the bedrock lag producing some rapid deglaciations or partial retreats by the following mechanism: at a glacial maximum the bedrock depression is relatively deep, so that any subsequent retreat is amplified by the increased ice melt at the lower surface elevations near the southern tip. These retreats are initiated at times of high summer insolation and coincide well with some of the observed deglaciations; however, there is still only partial agreement with the observed records. One drawback is that to achieve the retreats, the amplitude of the orbital forcing, k , has to be set unrealistically large which results in too much power at high frequencies (see Fig. 3*a*).

Another disconcerting feature of Fig. 1*b* is that some of the retreats do not appear to be complete enough; for instance, the present day model ice sheet extends from the Arctic shoreline to 65.5° N , and during the period 550–350 kyr BP the southern tip never retreats beyond 65° N . However, it is not known exactly how far the real ice sheets retreated in earlier interglacials; the minimum $\delta^{18}\text{O}$ values for these times in various deep-sea cores are sometimes less and sometimes greater than today's value. Ice volumes can also be inferred from eustatic changes in sea level measured by raised coral reefs, and during the last interglacial at ~ 125 kyr BP the sea level was $\sim 5 \text{ m}$ higher than at present¹³, suggesting that the Northern Hemisphere was at least as ice-free as today. Unfortunately, sea-level estimates for the earlier interglacials are more uncertain due to tectonic uplift, and show only that the ice volume must have been a small fraction of that during an ice age¹³.

At this point the model seemed to need more potential for complete deglaciations, but with less amplitude of the orbital forcing. This was attempted first by including topography in the model. The high ground on the Labrador–Ungava Plateau and/or Baffin Island has often been considered as important for the inception of ice sheets, and has been included in several

recent models^{14–16}. Figure 1*c* shows a run with the topographical term h'_0 in equation (3) roughly representing a present day section running south-southwest from Baffin Bay, over the high ground on Baffin Island, through Hudson Bay and on into the central USA; added to this is the residual depression from the last glaciation of up to 300 m centred on Hudson Bay¹⁷. The most important feature is the Baffin Island plateau with a peak at 70° N , and similar results can be obtained with this feature alone. By carefully adjusting the mean equilibrium line E so that it occasionally intersects the peak, small ice sheets can

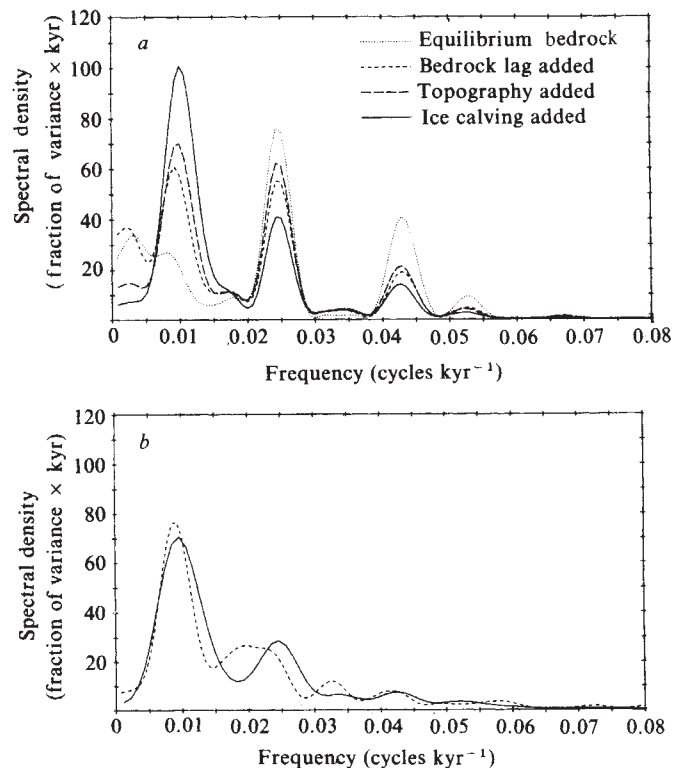
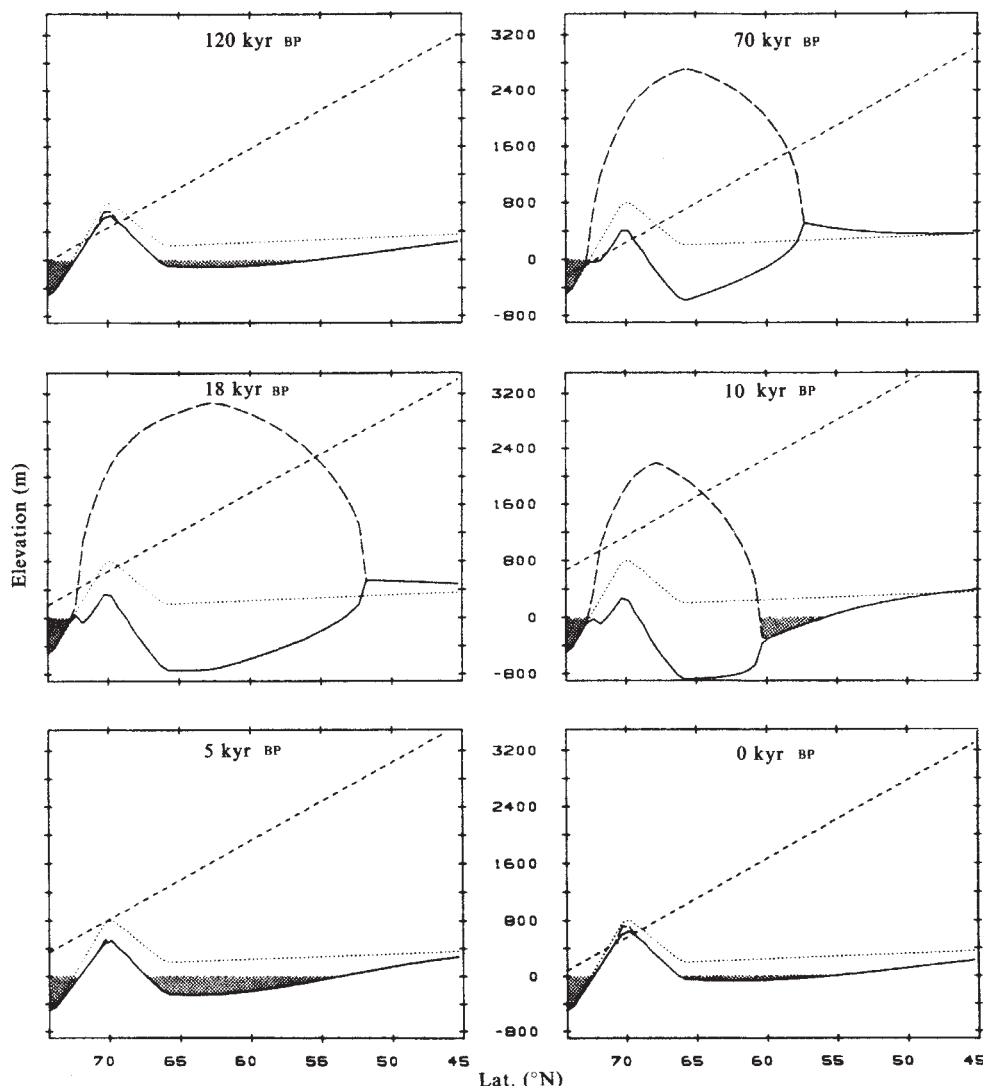


Fig. 3 *a*, Power spectral density for the model runs shown in Fig. 1. These were generated from time sequences of 350 points taken from each run at 2 kyr intervals, and detrended with a linear least-squares fit before computing autocorrelations. A Tukey window was used with the number of lags $\approx \frac{1}{3}$ of the total number of points. *b*, Power spectral density for the two deep-sea core records shown as the dotted curves in Figs 1 and 2*a*. These were computed as for the model curves above. Dashed curve from ref. 20; solid curve from ref. 29.

Fig. 4 Cross-sections versus latitude and height showing the ice sheet surface (long-dashed line) and the bed-rock surface (solid line) at various times in the model run of Fig. 1d. Also shown are the equilibrium line E (short-dashed line) and the undisturbed topography h'_0 (dotted line). The shaded regions show the extent of the invading water body that attacks the ice according to equation (4).



easily be initiated and grow out from the peak, whereas to the south E can still be high enough to place large ice sheets in jeopardy of complete deglaciations. Although the curve shows some improvement between ~ 350 and 0 kyr BP, the greater deglaciations were achieved only after careful tuning, especially in matching E to the topography. In addition an unrealistically large value for the ice/rock density ratio, $r = 0.4$, was required, and the required amplitude of the orbital forcing, k , still results in too much high-frequency power (see Fig. 3a). Even so, the period from 550 to 350 kyr BP still contains no complete deglaciations. Also the growths out of the interglacials at ~ 330 and 215 kyr BP are unrealistic, with anomalous partial retreats occurring at 285 and 170 kyr BP respectively.

These shortcomings indicated that an additional mechanism was required to yield a more robust model. It was noted that during the retreats in Fig. 1c coinciding with the observed deglaciations the southern tip of the ice sheet temporarily dropped far below sea level. Andrews³ has emphasized that rapid calving by vast proglacial lakes, which are known to have existed around the southern Laurentide ice sheet perimeter during the last deglaciation, may be the only plausible mechanism capable of producing the observed rapid rates of retreat. This view is supported by deep-sea core evidence of Ruddiman and McIntyre¹⁸, who infer massive discharge of icebergs into the North Atlantic after ~ 16 kyr BP. In addition marine incursions up the St Lawrence river and later into Hudson Bay at ~ 8 kyr BP are known to have greatly accelerated the retreat³. The calving process was included in the model by setting the mass balance G equal to a large negative value at ice sheet points between the tip and the first interior ice sheet grid point

able to block the invading body of water. The invading water, whose surface is taken to be at sea level, can penetrate into the ice sheet only as far as the ice columns can be floated by its hydrostatic head, so that

$$G(x_{i+1}) = -20 \text{ m yr}^{-1} \text{ if } \begin{cases} \rho_i h(x_i) < \rho_w (S - h'(x_i)) \\ \text{and } h'(x_{i+1}) < S \end{cases} \quad (4)$$

where ρ_w is the density of sea water, S the elevation of the current sea level and i the spatial grid index increasing into the ice sheet. Condition (4) is generally only satisfied, if at all, at the outermost ~ 50 – 150 km of the ice sheet when the tip is below the level S . The arbitrary value of -20 m yr^{-1} in equation (4) is comparable with estimates by Andrews³, but is much less than those suggested by analogies with non-floating calving glaciers today¹⁹.

Figure 1d shows a run including this calving mechanism and with the topography described above. Deglaciations, now augmented by equation (4), can occur with a reduced amplitude of the orbital forcing, and the overall agreement with the observed record is improved considerably. Interestingly the high insolation anomaly that triggers the deglaciation at 410 kyr BP is considerably weaker than those at some other times, for example around 175 and 85 kyr BP. Deglaciations do not occur at the latter times because the ice sheet is too small and the bedrock surface too high to allow calving.

There are still some disagreements with the observed records, especially before ~ 400 kyr BP; however, before this time the observed records themselves tend to diverge from each other^{1,20}. In fact, the apparent agreement can be improved by

selecting another deep-sea core record for comparison, as shown in Fig. 2a. For this model run the sea level S was made a linear function of ice volume and different initial conditions were chosen to show the relative insensitivity of the model with calving (in fact, much the same results can even be obtained with less realistic flat topography southward of 72° N). The effects of varying most of the model parameters have been investigated, and two examples are shown in Fig. 2b and c. Although these parameter changes are fairly substantial, the occurrence and phase of most of the main glacial cycles are preserved.

Figure 3 compares the spectra for the model curves in Fig. 1 with those of the deep-sea core records. A gradual increase in the ~100 kyr peak is seen as each new mechanism is added to the model. The spectrum with the calving mechanism has relative peak sizes in good agreement with those observed.

Figure 4 illustrates the last glacial cycle of the run in Fig. 1d. The ice sheet appears first on the northern peak at 120 kyr BP, then grows out to its maximum size which makes it susceptible to rapid deglaciation triggered by a period of high insolation, which begins just after 18 kyr BP. The calving mechanism sets in at ~11 kyr BP when the ice sheet tip is at 57° N, and enables the ice to vanish completely just after 5 kyr BP. By the present the bedrock has rebounded enough to support a small ice cap on the peak at 70° N. This is smaller than the present Barnes ice cap on Baffin Island, despite the model equilibrium line E being a few hundred metres below that suggested by present day estimates²¹. Although the amount of bedrock uplift since ~8 kyr BP is greater than observed⁷ by factors of ≈ 2 , the present residual depression agrees roughly with that implied by gravity anomalies¹⁷, and the present region below sea level south of ~66° N corresponds roughly with that of Hudson Bay.

As the simple model used here is tested mainly against just one type of observed record, any positive results should be regarded mainly as a guide for future work. Many feedback processes involving other parts of the climate system are neglected; for instance, the improvement in the model curves produced by the calving mechanism might also have been produced by any highly ablative mechanism that can be triggered only after the ice sheet has reached maximum size. One example is the freshening and shallowing of the oceanic mixed layer by massive ice sheet runoff, a self-amplifying mechanism thought primarily to reduce snowfall in winter²² and recently supported by deep-sea core evidence²³.

Equation (4) is an extremely simplified parameterization of the complex and not well understood mechanism of calving. An improved treatment would probably require the inclusion of east-west topography and ice sheet structure; with the present model equation (4) cannot distinguish between the known marine incursions from the east (up the St Lawrence river channel and into Hudson Bay, around each side of the Labrador-Ungava plateau), and the inland proglacial lakes that formed around the retreating Laurentide perimeter. Marine deposits from the past several thousand years are found only in the vicinity of the St Lawrence and the shores of Hudson Bay^{3,17}. This implies that the inland proglacial lakes formed mostly above sea level, in apparent disagreement with the model whose equatorward tip consistently drops a few hundred metres below sea level during deglaciation (Fig. 4). However, the basic mechanism represented by equation (4) is known to have induced rapid ice sheet disintegration when the sea invaded Hudson Bay at ~8 kyr BP (refs 3, 24), and is suspected of having induced past disintegrations of the West Antarctic ice sheet²⁵. The scenario hypothesized here could also apply to the Fennoscandian ice sheet, where the Scandinavian mountains form a northern plateau for ice sheet inception and the low-lying Baltic regions could have allowed the calving mechanism to be active during deglaciation.

Possible further work includes an extension of the model to three dimensions^{15,26}, improved treatment and comparison with data of calving by water bodies after ~16 kyr BP (refs 3, 18), inclusion of the elastic response of the lithosphere²⁷, and a test

of the results using a deep half-space asthenospheric model²⁸. In addition equation (2) can be replaced by coupling to a global seasonal climate model^{12,16}.

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Interaction of cryospheric forcings with rotational dynamics has consequences for ice ages

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Recent geological evidence^{1,2} suggests that several degrees of true polar wandering have occurred since the mid-Pliocene. This rotational instability is supported by dynamical calculations³ of the spin axis of a layered viscoelastic Earth, which has been subjected to the periodic forcings characteristic of the late Cenozoic ice age. We propose here that polar wandering, induced by the intrinsic response of the viscoelastic planet to such cryospheric forcings, may be the underlying cause for the termination of the present ice age epoch.

In the past decade there has been an increasing number of observations^{4–7} based on the correlation between the periodicities of the Earth's orbit and the time series analyses of the oxygen isotope data to suggest that there is a cause and effect relationship between the two systems. Nonetheless, it has been argued that internal stochastic mechanisms^{8–10} could be an important source for climatic variations. A new generation^{11–14} of climatic models has also emphasized the need to include the non-linear response of an ice sheet to changes in solar radiation in the attempt to understand better the mechanisms of ice ages. Here we pursue this theme and focus attention on the long-term effects of cryospheric forcings on producing secular motions of the Earth's spin axis, more commonly known as true polar wandering (TPW). The important consequences of TPW on the evolution of the climate system over a long time scale had long been appreciated¹⁵ and Gold¹⁶ had proposed that polar wandering is not only physically feasible but is to be expected for a viscoelastic planet.

First, we consider the recent findings¹⁻³ that suggest that there may be a connection between the late Cenozoic glaciation and TPW of $\sim 5^\circ$ in the past 10 Myr, as revealed by the recent reanalyses of the paths of palaeomagnetic poles. These data are based on the reconfirmation¹ that hotspots remain relatively fixed with respect to one another for the past 150 Myr. Calculations based on a layered viscoelastic Earth have supported this hypothesis that such an amount of polar wander relative to the entire mantle could, in fact, have occurred in response to the waxing and waning of large ice sheets in the past few million years. Over a shorter time scale, evidence¹⁷ from the past 75 years of International Latitude Service data also shows that TPW is taking place today. This phenomenon can be shown to be the direct consequence of the late Wisconsin deglaciation on the Earth's rotation^{18,19}. Thus evidence is accumulating to the effect that cryospheric forcings can strongly influence polar motions for a wide range of time scales. Our model calls on polar wander induced by ice ages ultimately to exceed a critical value of angular displacement which will dislodge the present climatic trend from its apparent equilibrium configuration. Such a finite-amplitude perturbation in the amplitude of rotation may cause the termination of ice ages. This event cannot be explained by Milankovitch's theory of astronomical forcings.

We now present our model which provides a physical description of polar wander produced by glaciation.

The expression of the conservation of the system angular momentum associated with the cryosphere and solid Earth is given by the Eulerian equations of motion, more commonly known in geophysics as the Liouville equation²⁰. For forcing functions from glacial loadings it takes the vector form

$$\frac{d}{dt}(\mathbf{J} \cdot \boldsymbol{\omega}) + \boldsymbol{\omega} \times \mathbf{J} \cdot \boldsymbol{\omega} = 0 \quad (1)$$

which is applicable for a body-fixed coordinate system rigidly attached to the Earth as a whole with its origin at the centre of mass and with the axes oriented such that the inertia tensor $\mathbf{J}$ initially is diagonal. $\boldsymbol{\omega}$ is the angular velocity vector.

The $\mathbf{J}$ tensor as a function of time t may be decomposed as follows,

$$\mathbf{J}(t) = I\delta_{ij} + C_{ij}(t) + I_{ij}(t) \quad (2)$$

where I is the diagonal inertia tensor of a bulging Earth in the absence of rotational fluctuations, $C_{ij}(t)$ is the component of the inertia tensor due to changes in the basic rotation rate, and $I_{ij}(t)$ is the contribution produced by transient viscous flow in the mantle and by shape deformation due to the glacial forcings. Variations in $I_{ij}(t)$ drive the spin axis away from its original position, whereas $C_{ij}(t)$ acts as a stabilizing agent in the readjustment of the rotational bulge as a consequence of the sudden change in the moment of inertia.

The nonlinear version of the Liouville equation must be used for polar displacements $> 20^\circ$ (ref. 19). But for the amount of polar drift found in the past 10 Myr (refs 1, 2) the linearized Liouville equations may be employed with sufficient accuracy. The solution has the simple Laplace transform domain form

$$\mathbf{m}(s) = \frac{\psi(s) + \phi(s)}{\left(1 + \frac{i}{\sigma_r} s\right)} \quad (3)$$

where $\mathbf{m} = (m_1, im_2)^T$ are the directions cosines of the rotation axis in the body-fixed coordinate system, s is a complex number and σ_r is the Chandler wobble frequency for the rigid Earth. $\psi(s)$ and $\phi(s) = (\phi_1, i\phi_2)^T$ respectively are the forcing functions arising from rotational deformation and the surface loading from ice masses. They have the explicit forms

$$\psi(s) = \frac{k_2^T(s, a)\mathbf{m}(s)}{k_t} \quad (4)$$

$$\phi_1(s) = \frac{I_{13}(s)}{C-A} + \frac{(sI_{23}(s) - \bar{I}_{23})}{\Omega(C-A)} \quad (5)$$

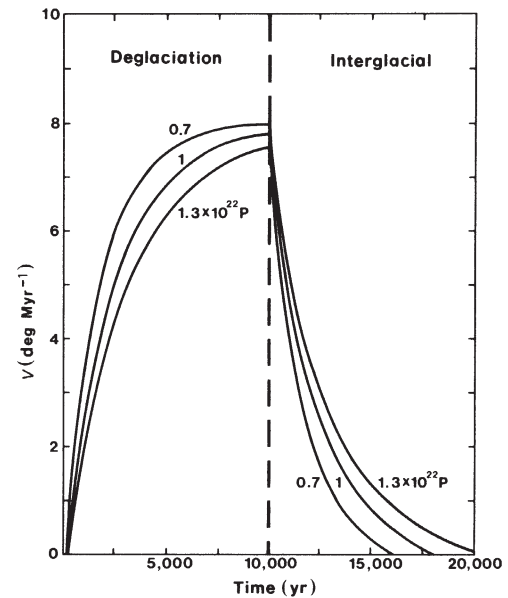


Fig. 1 Instantaneous speed of polar wandering during a glacial cycle. The length of time of the deglaciation phase is taken to be 10^4 yr, characteristic of the late Wisconsin. The speed of polar wander is taken to be positive when the direction of motion is towards the glaciating area. The end of the last deglaciation phase is around 5–6 kyr BP.

$$\phi_2(s) = \frac{I_{23}(s)}{C-A} - \frac{(sI_{13}(s) - \bar{I}_{13})}{\Omega(C-A)} \quad (6)$$

where Ω is the diurnal rotation rate, C and A are the axial and equatorial moments of inertia respectively and the overbars denote quantities at time $t = 0$. $k_2^T(s, a)$ is the tidal Love number, which represents the time-dependent behaviour of the gravitational potential in response to fluctuations in the rate of rotation, for angular order $l = 2$ evaluated at the surface ($r = a$) and k_t is the fluid Love number, which is a measure of the Earth's yield to centrifugal deformation over a time scale $0(10^9$ yr). Its value is about 0.95 (ref. 20).

For a given Earth model, $k_2(s, a)$, $I_{13}(s)$ and $I_{23}(s)$ can be obtained spectrally from the solution of a boundary value problem. Using a three-layer model, which consists of an elastic lithosphere, a viscoelastic mantle, and an inviscid core, we have obtained analytical expressions³ for the spectral decomposition of equations (4)–(6), which greatly facilitates the solution of the initial value problem of the polar displacement as a result of glacial forcing.

This three-layer model contains the essential physical ingredients needed for polar wander to occur. (1) The lithosphere is not isostatically compensated in the final state. Thus this residual elastic strain field produces a net polar displacement after the completion of a glaciation cycle. (2) Transient viscous flow in the mantle is influenced by the core and thereby produces another relaxation mode, whose strength of excitation is of the same order as that associated with the mantle mode.

The best available data which provide information on the actual time dependence of the extent of Pleistocene glaciation are the time series of oxygen isotope variability^{5,21}. To show that rapid rates of polar wander can occur at the glacial–interglacial boundary, we have conducted a series of model calculations for contributions from the two major ice sheets in the Northern Hemisphere, Laurentide in North America and Fennoscandia in Europe. A total mass of 2.4×10^{19} kg has been used in the loading and unloading process. For mathematical convenience a ramp-shaped function with an accretion time of 9×10^4 yr and with a disintegration time of 10^4 yr has been used to describe the climatic fluctuations³. This time-dependence can be transformed readily in the Laplace transform domain and used in equation (3). Figure 1 shows that with a range of

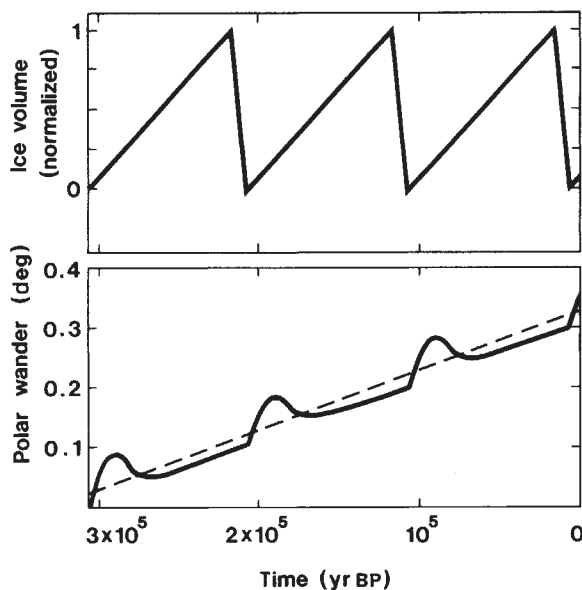


Fig. 2 Polar wander caused by cryospheric forcings. The forcing function is given at the top by the time-dependence of the ice volume, which has been normalized by an ice volume which is equivalent to a 70-m rise of the global sea level, a representative value of the unstable ice sheets in the Northern Hemisphere³³. The time of termination of the last deglaciation phase is taken to be 6 kyr BP. The response of the rotating viscoelastic Earth to such forcings takes the form of polar wandering. During this period the dashed line stands for the long-term polar displacement, whereas the solid line represents the short-term polar movements in response to glaciation and deglaciation.

mean mantle viscosities $0 (10^{22} \text{P})$ quite rapid rates of polar wander, up to 8°Myr^{-1} , which translates into around 80 cm yr^{-1} , can take place. However, as the period of deglaciation, $0 (10^4 \text{ yr})$, is short, the total polar displacement is only about 10 km. This amount, which involves polar motion relative to the mantle, is much smaller than changes in the obliquity, which involves polar motion relative to the fixed stars. This latter phenomenon arises from an external torque and may amount to $3\text{--}4^\circ$. Therefore, we would not expect our sporadic rapid motions of 80 cm yr^{-1} to have much of a visible signature on the ice coverage record. However, these rapid secular changes of the rotation axis may have some unforeseen effects on climatic variations²² between 10^3 and 10^4 yr .

Given that such rapid polar wander can occur in response to a single glaciation cycle, what then are the long range prospects for the late Cenozoic ice ages, which from the proxy climatic record seem to have reached a quasi steady-state trend consisting of a saw-tooth pattern for the past million years^{5,23,24}? To assess this possibility we have calculated the amount of polar wander, which has occurred since the mid-Pliocene from a forcing function which has been constructed on the basis of the proxy climatic record^{5,23}. Our calculations³ indicate that an average polar wander speed of 1°Myr^{-1} is attained for a viscosity of 10^{22}P .

These modelling results suggest that cryospheric forcings must be capable of producing TPW because of the basic physics due to the Earth's viscoelastic rheology¹⁶. If the present pattern of ice age cycles remains unchanged, then, barring any sudden changes of present plate motions, which might counteract the effects of TPW, Antarctica would begin to drift northward gradually by virtue of TPW. At the initial stages of this secular drift climatic warming may cause an increase of the melting from the Antarctic ice shelves. Over a short time scale, $0 (10^2\text{--}10^3 \text{ yr})$, continued weakening of the ice shelves²⁵ would then result in the ultimate collapse of the weakened portion of the Antarctic ice sheet with a consequent worldwide rise in the sea level of at least a few metres.

In the prevailing conditions of the Cenozoic ice ages, the entire mantle would shift relative to the spin axis. In this connection, data which have been combined from equatorial sediment facies²⁶ and palaeomagnetism²⁶, strongly support the occurrence of TPW, at least since the Cretaceous. They yield a motion of the rotation axis with a direction of about 25°E (towards Eastern Europe) in a reference frame in which the hotspots are fixed^{1,26}.

Secular motion of the Earth's spin axis relative to the geographical distribution of oceans and continents can have a profound influence on the future trend of the current ice age cycles. Milankovitch²⁷ considered that variations in the orbital configuration are critical for rapid growth of ice sheets in the Northern Hemisphere. The magnitude of the orbital variations are of a few degrees^{28,29}. Hence, if the present trend of polar wander were to exceed a certain threshold value, $0 (10^\circ)$, then such a large perturbation may bring about drastic changes in the climate system, such as changes in the role of the Coriolis forces, which control the patterns of oceanic currents to a large extent. Situated adjacent to the largest Northern Hemisphere ice sheets of the ice ages, the mid-latitude North Atlantic Ocean has, indeed, an important role in the Earth's recent climate history³⁰. One would expect, therefore, that under these circumstances of a shift in the direction of the Gulf Stream the response of the climate system to orbital variations might be altered drastically.

To demonstrate the feasibility of this mechanism, we have calculated in Fig. 2 the amount of TPW due to the periodic forcings from ice ages in the past $3 \times 10^5 \text{ yr}$. For a mean mantle viscosity of 10^{22}P , the speed of polar wander is about 1°Myr^{-1} . The saw-tooth curve of the Quaternary Ice Age shows clearly a 10^5-yr quasi-periodicity^{21,23}. The occurrence of an increasing trend in polar wandering in response to continual cryospheric forcings is a natural consequence of the rheological behaviour of the Earth. The time scale for this quasi-steady period is $0 (10 \text{ Myr})$, if the average polar wander speed is maintained steadily at $0 (1^\circ \text{Myr}^{-1})$ as shown in Fig. 2. Angular displacements of this magnitude would seem sufficient to displace the present configuration of continents and ocean circulations from the state of quasi-equilibrium which it now seems to enjoy. From then this new set of surface boundary conditions will not act in concert with astronomical forcings to produce large scale continental glaciation.

Finally, for each epoch of ice ages, the magnitude of the cryospheric forcings is governed by the particular geographical distribution of continental platforms, which may be linked with the possible time-dependent nature of mantle convection. For the Cenozoic ice age, whose inception is still fraught with uncertainties^{31,32}, we may expect this present periodic phase to persist for another few million years, before it undergoes self-destruction by TPW.

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Picrites as parental magma of MORB-type tholeiites

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Recent experimental work on peridotites and basalts^{1–3} and a reappraisal of ophiolite complexes⁴ supports the concept that basalts are derived from picritic parents^{5–7}. The occurrence of a density minimum in the liquid line of descent^{8–10} explains why mid-ocean ridge tholeiites fall in a restricted compositional range, and parental picrites can only be found in the exceptional case when eruption is not yet controlled by a steady-state magma chamber^{7,11}. We present here a preliminary account of a natural example of such a case from the Caribbean.

The Cretaceous Curaçao Lava Formation is a more than 5-km thick succession of submarine basalts with more than 20 flows of picritic pillow basalts in its lower part. The base of the succession is not exposed. The picrites are associated with variolitic basalts and olivine-phyric tholeiites. Olivine-phyric and plagioclase-pyroxene-phyric tholeiites make up the bulk of the formation, the latter predominating in the upper part. Composition of glass and of olivine megacrysts and phenocrysts in the picrites indicates that the olivine-phyric tholeiites were derived by fractionation of olivine from a picritic parent with at least 17.5 wt% MgO. The plagioclase-pyroxene-phyric tholeiites are derived by further fractionation of olivine, plagioclase and pyroxene. The picritic basalts in the section have 19–31 wt% MgO, which shows that part of their olivine is cumulative. Model calculations suggest that the parent results from 30% partial melting of a mantle of pyrolytic composition.

The variolitic basalts associated with the picrites in the lower part of the unit, pose a problem. Major element chemistry of these rocks show that they are not related to the other basalts by low pressure fractionation. However, incompatible element ratios are roughly similar for all basalts of the succession, which suggests that they are derived from the same mantle source.

The island of Curaçao (positioned between 12°2' N and 12°23'30" N, and 69°10' W and 68°44'30" W) is the larger of an east-west-trending group of small islands in the southern Caribbean. Geophysical and geological evidence suggests that the islands are underlain by a basement of oceanic crust of pre-Campanian age¹². The Curaçao Lava Formation is the lowermost unit exposed in Curaçao¹³. It consists almost entirely of volcanic rocks but contains one intercalation of a few metres thickness of pelagic sediments in its upper part. These contain an ammonite fauna of middle Albian age¹⁴. The Curaçao Lava Formation consists largely of pillowed flows. They are virtually the only rock-type in the lower half of the formation; in the upper half they are still predominant, but alternate with reworked hyaloclastites and dolerite sills and dykes. A sheeted dyke complex is not exposed.

Whole-rock major elements used here have been determined by X-ray fluorescence spectrometry (University of Amsterdam); rare earth elements (REE) by instrumental neutron activation analysis (Inter-university Reactor Institute, Delft); glass and minerals were analysed with an electron microprobe (Instituut voor Ardwetenschappen, Vrije Universiteit, Amsterdam).

Most picrites contain between 19 and 22 wt% MgO (Fig. 1). These flows all have pillow structure and vary in thickness from a few tens of metres to roughly 100 m. Pillow shape and size is equal to those of the tholeiites. The rocks consist of more or less euhedral olivine phenocrysts (Fo_{86.5–87.4}) of up to 2.5 mm in size, embedded in a groundmass of glass and skeletal quench crystals of olivine and clinopyroxene. The amount of glass decreases rapidly from rim to core of the pillows. Chrome-rich spinel occurs as an accessory constituent. The olivine phenocrysts are unzoned. They make up 27–32 vol.% of the lavas. Gravitational settling of olivine within a flow or within a single pillow is negligible, and the proportion of olivine always falls within these limits. Glass from the pillow margin of one of these picrites (79KV615, Table 1) plots on the Fo₈₇ control line and within the field of the olivine-phyric tholeiites (Fig. 1). This glass (MgO = 10.6%, FeO = 8.6%) is similar in composition to the most primitive liquids erupted at mid-ocean ridges. It is in equilibrium with the Fo₈₇ phenocrysts, using a K_D for partitioning Mg and Fe between olivine and melt of 0.3 (ref. 15). This implies that the olivine-phyric tholeiites are derived from a parent with a MgO-content ≥ 10.6 by equilibrium crystallization of olivine. The occurrence of strongly resorbed olivine mega-

Table 1 Representative analyses of picrites and basalts of the Curaçao Lava Formation

	Picrite 79KV615	Glass picrite 79KV615	Olivine phenocryst 79KV615	Olivine-phyric tholeiite 79Be248	Plagioclase- pyroxene- phyric tholeiite Be2041	Variolitic basalt 79BK207
SiO ₂	45.83	49.7	40.6	50.61	51.22	51.46
TiO ₂	0.68	0.95		0.86	1.35	0.81
Al ₂ O ₃	9.98	13.8		14.61	13.99	12.97
Fe ₂ O ₃	0.92	0.86		0.88	1.07	0.81
FeO	9.18	8.62	11.21	8.75	10.67	8.05
MnO	0.17	0.16	0.10	0.16	0.2	0.19
MgO	20.00	10.6	47.33	9.37	6.61	8.66
CaO	8.6	11.9	0.3	11.87	9.31	14.81
Na ₂ O	1.12	1.6		1.7	3.75	1.2
K ₂ O	0.09	0.07		0.13	0.56	0.03
P ₂ O ₅	0.05			0.06	0.1	0.06
Cr ₂ O ₃	0.27	0.08		0.05	0.01	0.08
NiO	0.11		0.46	0.02		0.03
Mg/(Mg + Fe)	0.80	0.69	0.88	0.66	0.52	0.66
La/Sm	0.9			0.84	0.95	1.07

Mg/(Mg+Fe) in atomic ratio; Fe₂O₃ = 0.1 FeO

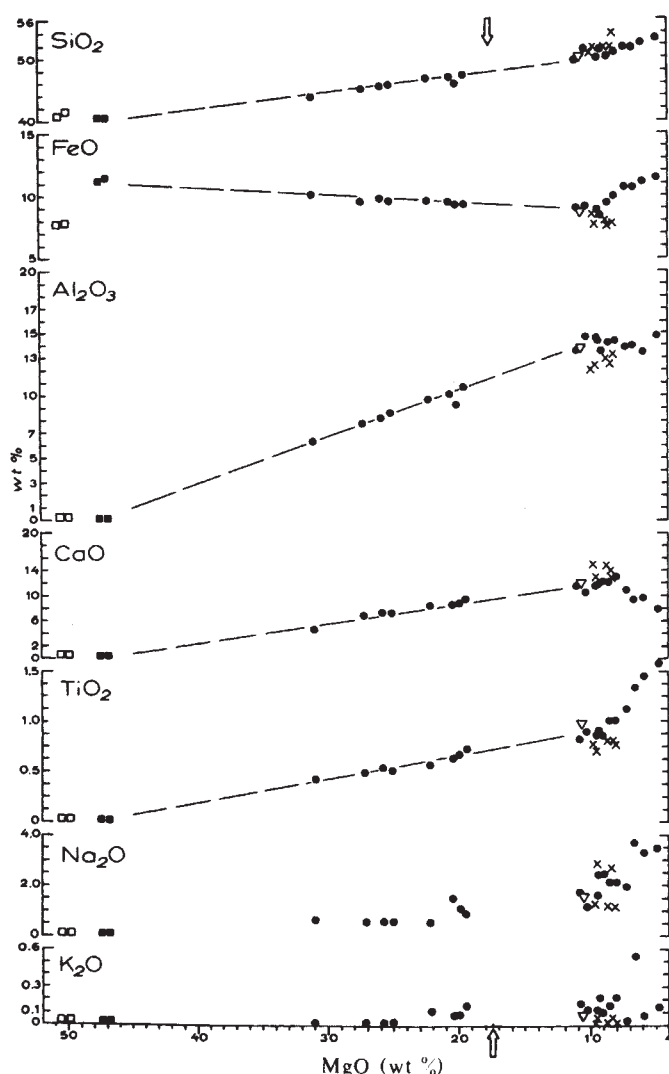


Fig. 1 MgO-variation diagram of Curaçaoan picrites and basalts. ●, Picrites, olivine-phyric tholeiites and plagioclase-pyroxene-phyric tholeiites; ×, variolitic basalts; ■, olivine phenocrysts of picrites (Fo_{86.5-87.4}); □, olivine megacrysts (Fo₉₀ and Fo_{91.4}); △, glass of picrite 79KV615. Arrows indicate MgO percentage of calculated parent liquid using a K_D (ref. 15) of 0.3 and $Fe_2O_3 = 0.1$ FeO.

crystals up to 5 mm long and with a composition up to Fo 91.4 (Table 2, Fig. 1) in three of the picritic flows brings us nearer to a conclusion about the composition of the parent. Assuming that they represent early formed, non-equilibrated phenocrysts, the composition of the melt in which they formed can be calculated using Roeder and Emslie's K_D of 0.3 and the MgO variation diagram (Fig. 1). This melt (Table 2) has about 17.5% MgO. A slightly higher K_D would give a parent within the range of the pillowed picrites (MgO 19–22%); the use of an oxidation ratio lower than the value assumed for the picrites (0.1) has the same effect: taking total Fe as FeO gives a parent with 19.9% MgO.

Accumulation of olivine is evident in the picrites with a MgO content of 25–31 wt%. These olivines are rounded and partly resorbed. In the most Mg-rich flows olivine forms up to 60 vol.%.

The control line of the MgO variation diagram (Fig. 1) changes in slope between 8 and 9 wt% MgO, indicating that fractionation of plagioclase and pyroxene defines the composition of the more evolved tholeiites. The variolitic basalts, with a MgO wt% similar to that of the olivine-phyric tholeiites, systematically plot outside the fractionation trend of most of the volcanics of the Curaçao Lava Formation.

The chondrite-normalized REE patterns (Fig. 2) of picrites, olivine-phyric tholeiites and plagioclase-pyroxene-phyric tholeiites are consistent with consanguinity of the suite. They are basically flat (T-type MORB; La/Sm_N = 0.8–1.0). From the MgO/Sm ratio the REE content of the parent can be estimated to be about 6 times chondritic^{1,16}, the parent magma would represent about 30% batch partial melting. This amount of melting would leave a residue of olivine and orthopyroxene only. Although residual rocks of the Curaçaoan succession are not known, a model calculation is given in Table 2, using the olivine megacrysts (Fo_{91.4}) of sample 79Be148, and an orthopyroxene of Green *et al.*¹. A small amount (0.5%) of a chrome-rich spinel from sample 79KV615 is added to the residue, mainly to raise the Cr₂O₃-content of the source to acceptable values. The model calculations are furthermore constrained by: (1) composition of the residue, that must fall within the field of that of natural, mantle derived periodites¹⁷; (2) 3–4 wt% CaO and 4–5 wt% Al₂O₃ in the source composition, that is compositions similar to previous estimates of upper mantle pyrolite¹⁸. The first constraint, in combination with 30% partial melt, mainly defines the proportions of the residual phases as approximately 80% olivine and 20% orthopyroxene. A much higher amount of orthopyroxene would give a residue too high in SiO₂ and too low in MgO. The second constraint necessitates the selection of an Al₂O₃-rich orthopyroxene. The residual orthopyroxene of

Table 2 Parental liquid of Curaçaoan basalts, and model calculation of the source

	Parental liquid, recalculated at 100%	Olivine- megacryst Fo _{91.4} 79Be148	Orthopyroxene*	Spinel 79KV615	Residue F = 0.3 78% ol. 21.5% opx 0.5% spinel	Source	Source of DSDP3-18
SiO ₂	48.33	40.7	54.8		43.53	45.00	45.00
TiO ₂	0.74			0.49		0.22	0.17
Al ₂ O ₃	11.35		5.5	19.90	1.28	4.35	4.4
Fe ₂ O ₃	0.94						
FeO	9.35	7.8	4.9	18.2	7.22	8.13†	7.6
MnO	0.17	0.12			0.09	0.11	0.11
MgO	17.70	50.55	32.0	14.3	46.38	37.61	38.8
CaO	9.57	0.2	2.8		0.76	3.46	3.4
Na ₂ O	1.34					0.41	0.4
K ₂ O	0.11					0.03	0.003
P ₂ O ₅	0.06					0.02	
Cr ₂ O ₃	0.25	0.1	1.2	45.7	0.56	0.47	0.45
NiO	0.09	0.38	0.05	0.19	0.31	0.25	0.26

* From ref. 1.

† FeO total.

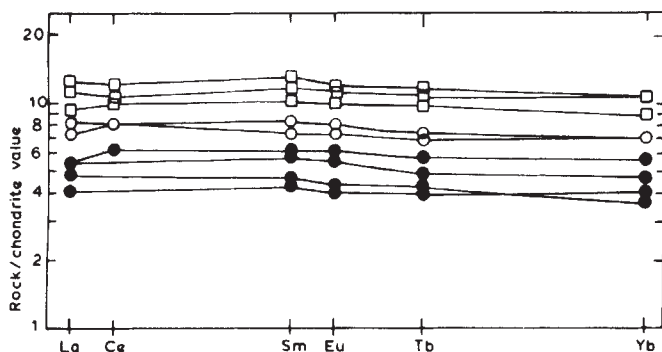


Fig. 2 Chondrite-normalized REE-patterns of representative basalts of the Curaçao Lava Formation. ●, Picrites; ○, olivine-phyric tholeiites; □, plagioclase-pyroxene-phyric tholeiites. MgO-content of the basalts are, from base to top: 27.5; 25.1; 21.0; 20.7; 11.0; 10.3; 8.3; 6.0; 4.9.

Green *et al.*¹ best fits our needs, and gives a source (Table 2), which is in good agreement with that of earlier calculations^{1,18}. The main difference with the source of DSDP 3-18 (ref. 1) is the much higher K₂O-content, reflecting the transitional nature of the Curaçaoan basalts.

The common occurrence of picritic flows in the lower part of the Curaçao Lava Formation suggests that, at this stage of development of the volcanic unit, magma reservoirs are small. They enlarge as the volcanic pile builds up, and the height of the magma column between reservoir and surface becomes such that the dense picritic liquid can no longer extrude. Fractionation of olivine, and, at a later stage, of pyroxene and plagioclase in these reservoirs, provide the less dense tholeiites of the upper half of the formation. These considerations suggest that the Curaçao Lava Formation was formed because of the initiation of a new system, rather than being a fragment of oceanic crust fed by a steady-state magma chamber. The new system is not the opening of the Caribbean, as this occurs much earlier¹⁹. Considering the close association of the unit in space and time with volcanic sequences of island-arc origin (late Albian to Coniacian on the neighbouring islands of Aruba and Bonaire²⁰), we consider the unit to be the base of an island-arc succession formed either during the initiation of subduction²¹, or because of lengthening of an existing arc at a trench-trench-ridge triple junction. These models will be discussed more fully elsewhere.

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Seabed-wave resonance and sand bar growth

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The interaction of surface water waves with undulating sea-bed topography is of fundamental importance to coastal engineers and sedimentologists. For example, wave reflection from submerged bars on beaches may provide a mechanism for protecting the beach from further wave attack. Additionally the interaction of surface water waves with other tidally or wave generated bedforms, such as sandwaves on offshore banks, may modify wave climates on adjacent coastlines. While there have been numerous studies of the interaction of sea waves with engineering structures, such as breakwaters, piers, and jetties, there have been comparatively few investigations of wave interactions with naturally occurring bedforms. Predictions have recently been made of the amount of wave energy reflected as a result of resonant interactions between surface water waves and undulating sea-bed topography but with no supporting experimental proof. I describe here preliminary results from what are believed to be the first published laboratory measurements of resonant interactions between surface water waves and submerged bars which show that significant and large amounts of wave energy may be reflected and that these reflections are brought about by resonant interactions between surface-water waves and the bedforms. In particular, at resonance, incident surface water wavelengths are approximately twice the bedform wavelengths. These results have implications not only in terms of wave reflection from naturally occurring bedforms, say bars on beaches, but also for sediment transport processes in general.

Davies^{1,2} has used linear perturbation theory to show that to a first approximation, wave reflection from a finite number of submerged sinusoidal bars, having small amplitude and on an otherwise plane bed, is given by

$$K_r = \frac{a_r}{a_i} = \frac{2bk}{\{2kh + \sinh(2kh)\}} \left(\frac{2k}{l} \right) \frac{\left| \sin \left(\frac{2k}{l} m \pi \right) \right|}{\left(\frac{2k}{l} \right)^2 - 1} \quad (1)$$

where K_r is the wave reflection coefficient, a_r and a_i are the reflected and incident wave amplitudes respectively, well away from the region of bedforms, b is the bar amplitude, h is the water depth, m is the number of bars and k and l are the free surface and bar wavenumbers. Here $k = 2\pi/L$ and $l = 2\pi/L_b$, where L and L_b are surface and bar wavelengths respectively. In the theory it was assumed that the wave crests were parallel to the bars and that the flow was non-separating. Additionally, certain constraints were imposed on the result (equation (1)) which included the following: a_r/k , a_i/h , $a_i/k^2 h^3$, bl , b/h and $bk \ll 1$. Furthermore, unless the solution is corrected for wave attenuation across the bars (see later) it is required that $|a_r/a_i| \ll 1$.

Following Davies¹, the result in equation (1) may be considered in two parts the second of which we shall denote as

$$H \left(\frac{2k}{l} \right) = \left(\frac{2k}{l} \right) \frac{\left| \sin \left(\frac{2k}{l} m \pi \right) \right|}{\left(\frac{2k}{l} \right)^2 - 1} \quad (2)$$

Equation (2) illustrates that for a given number of bars (m),

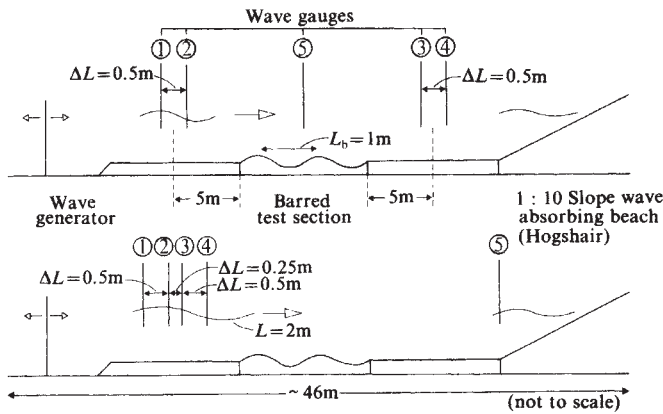


Fig. 1 Positions of gauges, in relation to barred test section and wave absorbing beach, for two main types of measurements. Typical values of the wave gauge spacing ΔL are also shown.

the wave reflection coefficient is oscillatory in $2k/l$, that is the quotient of twice the surface wavenumber and the bed wavenumber. The reflection coefficient is also resonant in the region $2k/l \approx 1$ and, at $2k/l = 1$ itself, $H(2k/l) = m\pi/2$ which suggests that peak reflection coefficients are linearly dependent on the number of bars present.

To test Davies^{1,2} theoretical predictions and in particular equations (1) and (2), detailed measurements of wave reflection from submerged bars were carried out using the $45.72 \times 0.91 \times 0.91$ m wave tank facility at the Coastal Engineering Research Center, Virginia. A 10-m long test section consisting of 10×1 m wavelength, 0.05-m amplitude sinusoidal bars was constructed in the tank and set in a false bottom. The barred test section was situated approximately midway between a hydraulically driven piston type wave generator, at one end of the tank, and a 1:10 slope wave absorbing beach at the other. Water surface elevations were measured using standard parallel-wire resistance type wave gauges and wave reflection coefficients determined using the method of Goda and Suzuki³.

Two pairs of gauges and a single gauge were used to make two types of measurement of which some preliminary results are shown here; first incident and transmitted wave conditions were measured with one gauge pair 5 m on the up-wave side of the bars and the second gauge pair 5 m on the down-wave side. The remaining gauge was positioned midway along the test section. The up-wave gauge pair thus gave information on wave reflection from the bars while the second gauge pair provided data on the transmitted wave heights and the amount of wave energy reflected from the beach. In the second type of measurement two pairs of gauges were moved along the tank

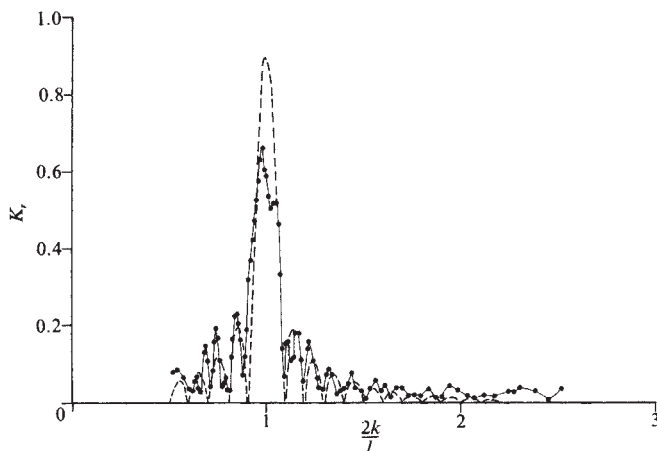


Fig. 2 Comparisons of measured (●) and predicted¹ values (---) of the reflection coefficient, K_r , as a function of the wavenumber ratio $2k/l$, for $b/h = 0.16$ and $m = 10$ bars.

in such a way as to give surface elevation data every 0.25 m and to determine how wave reflection varied throughout the tank, first from the barred test section and finally from the beach. The remaining gauge was positioned at the end of the tank at the foot of the beach. These experimental arrangements are illustrated in Fig. 1.

With the bar wavelength, L_b , fixed at 1 m, incident surface water wavelengths were varied over a range giving $0.5 \leq 2k/l \leq 2.5$, by varying the wave period in steps of 0.01 s. For the results shown here, water depths were varied to give bar amplitude-water depth ratios, b/h , in the range $0.08 \leq b/h \leq 0.16$. Thus, good resolution, in non-dimensional wavenumber space $2k/l$, of the order of 0.01, enabled detailed investigations to be made of the oscillatory nature of the wave reflection coefficient and of the resonant interaction peaks. These tests were carried out using small amplitude monochromatic waves only and with wave steepness values which complied with the scaling laws given by Davies¹ and shown previously.

For surface water wavelengths approximately twice the bar wavelength, strong resonant interactions were observed leading

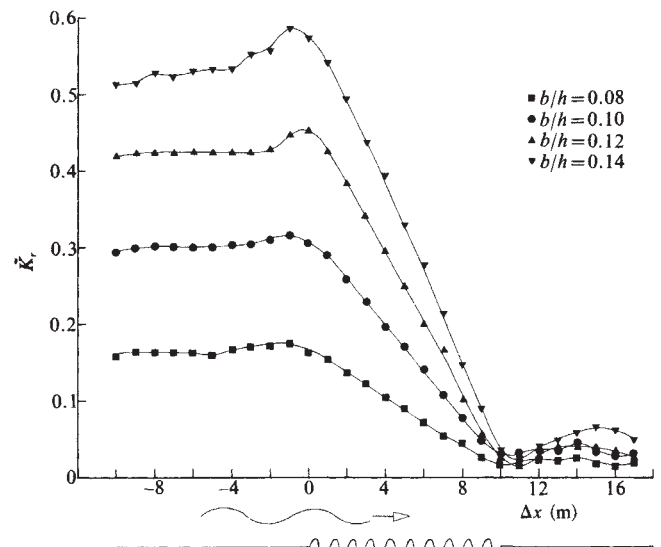


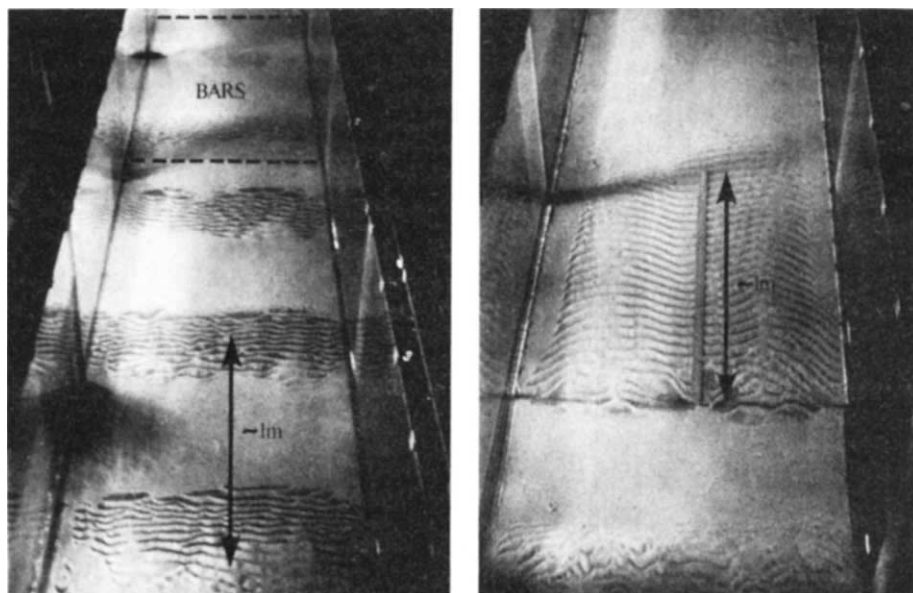
Fig. 3 The reflection coefficient, $\tilde{K}_r$, as a function of distance along the wave tank, Δx , and different ratios of bar amplitude to water depth, b/h , for $m = 10$ bars. Note that the origin is taken as the position of zero elevation of the first bar and that $\tilde{K}_r \rightarrow K_r$ for $-\Delta x/h > 10$.

to large reflection coefficients (in some cases as large as $K_r = 0.8$) and dramatic standing or partial standing wave patterns on the up-wave side of the bars. On the down-wave side of the bars the standing wave pattern gave way to progressive waves leaving the test section and travelling towards the wave absorbing beach.

Figure 2 shows the variation of wave reflection coefficient, K_r , with the wavenumber ratio $2k/l$ for 10 bars and a bar amplitude-water depth ratio of $b/h = 0.16$ ($h = 31.3$ cm). Also shown are the first-order predictions from Davies^{1,2}, uncorrected for the effects of wave attenuation as the incident waves propagate over and are reflected by the bars. If the theoretical results are corrected by the method proposed by Davies¹, to relax the condition $|a_r/a_i| \ll 1$ and to give a more realistic result allowing for wave attenuation, the predicted peak value of K_r at $2k/l \approx 1$ is $K_r = 0.75$, which is in quite close agreement with the measured value.

A striking feature of these results is the large resonant interaction peak at $2k/l \approx 1$, with peak reflection coefficients of ~ 0.65 , and the oscillatory nature of K_r in respect of $2k/l$. Figure 3 shows the results from measurements of the reflection coefficient at resonance, at different positions, Δx , along the tank and throughout the barred test section. Note that this is a modified reflection coefficient $\tilde{K}_r$, such that $\tilde{K}_r \rightarrow K_r$ for $-\Delta x/h > 10$. Measurements are shown for bar amplitude-water

Fig. 4 Left, ripple patches with a 1-m spacing formed beneath a partial standing wave on the up-wave side of 2×1 m wavelength bars. The bar amplitude is 5 cm and for these observations the water depth was 15.6 cm and the wave reflection coefficient was $K_r \approx 0.34$. The largest ripples were ~ 1.5 cm high with wavelengths of about 5.5 cm. Right, a continuous sheet of ripples formed down-wave of the reflecting bar system. Ripple heights were of the order of 1.0-cm with wavelengths of about 4 cm.



depth ratios in the range $0.08 \leq b/h \leq 0.14$ that is $35.7 \leq h \leq 62.5$ cm. The resonant wave periods varied from 1.17 to 1.28s, dependent on actual water depth, and were usually within 0.02s of the predicted values. These results indicate that on the up-wave side of the bars the reflection coefficient, $\bar{K}_r \rightarrow K_r$, is more or less constant and rises to a peak value within a few water depths of the bars before falling, linearly throughout the test section, to values of order 0.05 or less, which is the reflection from the beach alone. While only preliminary measurements for 10 bars are shown here, later measurements on 4, 2 and 1 bars have shown similar trends to those given in Figs 2 and 3. These and other observations are to be reported more fully elsewhere.

Davies^{1,2} has suggested that as a result of the partial standing wave which forms up-wave from a reflecting bar system, the pattern of wave orbital motions near the bed may lead to areas of preferential erosion and deposition of sediment. Potentially, at least, this provides a mechanism for bars to grow in the up-wave direction.

To confirm this result fine sand of about 235- μ m mean diameter was sprinkled in a thin uniform layer throughout the barred test section (with 2 bars only) and for about 2–3 m on either side of it. Waves were started from rest and the wave amplitude increased until sediment motion was initiated. Sediment movement was then observed for a resonant wave reflection condition ($K_r \approx 0.34$) and the evolution of ripple patches recorded on the up-wave and down-wave side of the bars. Figure 4 shows results from these experiments. The important features are the formation of ripple patches with a 1-m spacing on the up-wave side of the bars (Fig. 4a) and the appearance of a more or less continuous sheet of ripples on the down-wave side (Fig. 4b). Both these features are associated with a 2-m surface wavelength. In Fig. 4a erosion and ripple formation was observed to occur beneath the nodes of surface elevation of the partial standing wave. With increasing time ripple heights were observed to grow on the up-wave side of each patch in such a way as to bring about an accumulation of material approximately midway between node and antinode and roughly in the position where bar crest formation would be expected to occur. These results confirm that a potential bar-growth mechanism occurs up-wave of the bars (Fig. 4a), but not on the down-wave side (Fig. 4b), and while these results cannot be extended unambiguously to the case of naturally occurring sand bars on an erodible bed, there is strong evidence to suggest that such a mechanism may occur in practice. Note that Nielsen⁴ also found evidence of bar growth beneath standing waves (not generated by bars) and in particular that fine sand (80 μ m), moving mainly in suspension, accumulated

directly under the antinodes. The results described here are for coarser material moving principally as bedload and the resulting distribution of sediment is governed more by velocities near the bed and less by the convection cells described by Nielsen.

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Downcore variation in sediment organic nitrogen

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An historical record of natural (pre-cultural) and anthropogenic (influenced by man's activities) chemical inputs to the environment is contained in accumulating sediment deposits and is commonly expressed as a depositional flux¹. This flux is traditionally calculated as the product of the sediment accumulation rate and the concentration of a substance in the sediment¹. Previous work has quantified depositional fluxes of metals and nutrients by assuming that the vertical distribution of these substances in sediments is unaffected by post-depositional migration, chemical reaction or porosity variations^{2–10}. We present here a procedure for calculating the historical depositional flux (loading record) of a substance which undergoes post-depositional chemical reaction in a compacting sediment column. The method is demonstrated by calculating the loading record of organic nitrogen at one locality in Lake Erie.

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Organic nitrogen was selected for study because its vertical distribution in Lake Erie sediments (41°42.0' N; 82°18.8' W) depends solely on the depositional flux of particulate organic nitrogen and the post-depositional rate of microbial decomposition of organic nitrogen; nitrogen does not participate in mineral equilibrium reactions in Lake Erie sediments. Microbial degradation of particulate organic nitrogen compounds converts their contained nitrogen directly to ammonium¹¹. We have assumed that the destruction of particulate organic nitrogen may be quantified by measuring net ammonium production. Because some of the produced ammonium may be converted to other nitrogen species, the assumed rate of decomposition of particulate organic nitrogen is a minimum. As a result, the calculated loading record for organic nitrogen represents the minimum organic nitrogen loading record at this locality.

The rate of nitrogen loss from sediment solids (or gain by interstitial water) is proportional to the concentration of particulate organic nitrogen present¹¹⁻¹³. This is illustrated with data from the Lake Erie study site in Fig. 1. At any depth z (measured positively downwards) this may be expressed as

$$R(z) = -k(z)N_p(z) \quad (1)$$

where $N_p(z)$ is the concentration of particulate organic nitrogen at depth z (mol N per mass sediment particles), $k(z)$ is the apparent rate 'constant' (time⁻¹) at depth z , and $R(z)$ is the rate of ammonium production at depth z . Note that $k(z)$ is not a constant. It is a function of the nature of the particulate organic nitrogen undergoing decay, and, consequently, depth in the sediment. In addition, $k(z)$ represents the resultant of many chemical reactions. Nevertheless, it is convenient to think of $k(z)$ as an apparent integrated rate constant at depth z . It is implicitly assumed that all particulate organic nitrogen present can be utilized by bacteria. The continuity equation describing the change in nitrogen content in a mass of sediment particles travelling downward from the sediment-water interface is

$$\frac{dN_p}{dt}(z, t) = \frac{\partial N_p}{\partial t} + \omega(z) \frac{\partial N_p}{\partial z} = R(z) \quad (2)$$

where $\omega(z)$ is the downward velocity of sediment particles at depth z due to sedimentation. ω is given by

$$\omega(z) = q/\rho(z) \quad (3)$$

where q (assumed constant) is the mass flux of sediment particles to the sediment-water interface (mass/area/time) and $\rho(z)$ is the mass of sediment particles contained in a volume of sediment at depth z :

$$\rho(z) = \rho_s(1 - \phi(z)) \quad (4)$$

where ρ_s is the density of sediment particles and $\phi(z)$ is the ratio of void volume to total sediment volume at depth z . Assuming a constant flux of particles to the sediment-water

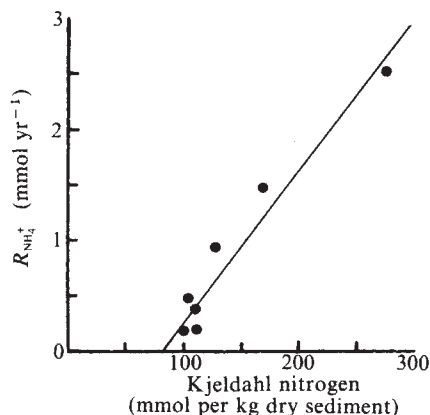


Fig. 1 Rate of ammonium production versus Kjeldahl nitrogen for sediments from the Lake Erie locality. Data from ref. 13.

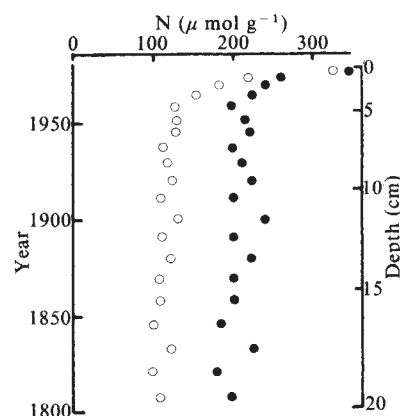


Fig. 2 Present-day particulate organic nitrogen concentration (N_p , ○) and calculated particulate organic nitrogen concentration at the time of deposition (N_{po} , ●) plotted against depth and time of deposition at the coring locality.

interface and steady-state compaction, the solution to equations (1)–(4) is

$$N_p(z, t) = N_{po} \exp \left[-\frac{1}{q} \int_0^z k(z) \rho(z) dz \right] \quad (5)$$

where t = present time and

$$N_{po} = N_p \left(t - \frac{1}{q} \int_0^z \rho(z) dz \right)$$

N_{po} is the concentration of particulate organic nitrogen at the time of deposition. The historical record of N_{po} can be calculated using equations (1) and (5). Measured values of $N_p(z)$ (as total Kjeldahl nitrogen), $R(z)$ (as ammonium production)¹³, $\phi(z)$ (calculated from water content and ρ_s), and q (by ²¹⁰Pb; $q = 0.077 \text{ g cm}^{-2} \text{ yr}^{-1}$) (J. A. Robbins, personal communication) were used in the calculation. The values of $R(z)$ represent net rates for ammonium production. Conversion of produced ammonium to other nitrogen species or adsorption onto sediment particles¹⁴ were not accounted for in the production rate experiments. The results of this calculation are shown in Fig. 2 along with the measured present day concentration of particulate organic nitrogen, N_p .

The data presented in Fig. 2 indicate that the loading of particulate organic nitrogen to sediments at the coring locality has increased since ~1950. Scatter in the data preclude a detailed examination of particulate organic nitrogen flux before this date. The results are in reasonable temporal agreement with estimates of phosphorus input to Lake Erie¹⁵. In the early nineteenth century (1820–60), the flux of particulate organic nitrogen qN_{po} was $\sim 0.165 \text{ mol m}^{-2} \text{ yr}^{-1}$. In the recent past (1974–77), the flux of particulate organic nitrogen was $\sim 0.285 \text{ mol m}^{-2} \text{ yr}^{-1}$. This gives a current ratio of anthropogenic to natural nitrogen loading of ~ 0.73 . This result is substantially less than estimates of this ratio made by Kemp *et al.*⁵. These workers calculated the average present ratio of anthropogenic to natural nitrogen loading to Lake Erie sediments as $\sim 1.79 \pm 0.78$. They did not consider decomposition of organic matter containing nitrogen. In fact, the values of N_{po} calculated here are minima. The rates of ammonium production used do not include any conversion of ammonium to nitrate or nitrogen gas. Further, loss of particulate organic nitrogen due to oxygen and nitrate reduction in the uppermost portion of the sediment column is completely neglected¹³. Inclusion of these reactions would increase N_{po} and lower the anthropogenic to nitrogen loading ratio. The omission of the decomposition of particulate organic nitrogen in the calculation of organic nitrogen loading to sediments will increase the apparent ratio of anthropogenic to natural nitrogen loading. For example, the application of the methodology used by Kemp *et al.*⁵ to our organic nitrogen data yields a current anthropogenic to natural nitrogen loading ratio of 1.99. The neglect of organic decomposition in estimates of

man's effect on the lake's nitrogen budget increases the apparent anthropogenic contribution by ~2.7 times.

The method outlined above for incorporating decomposition should be tested at other sites and, with appropriate modifications, applied to other reactive components such as phosphorus and carbon. This approach could produce significantly different nutrient budgets in many environments and may lead to a reevaluation of some management criteria.

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Eocene to Oligocene benthic foraminiferal isotopic record in the Bay of Biscay

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We present here oxygen and carbon isotopic records of Eocene to Oligocene benthic foraminifera from two Bay of Biscay Deep Sea Drilling Project (DSDP) sites (119 and 401). $\delta^{18}\text{O}$ of benthic foraminifera increases 1.9‰ from a middle Eocene minimum (Zones P10–P11) to an earliest Oligocene maximum (Zone NP21). Approximately 1.4‰ of the increase in benthic foraminiferal $\delta^{18}\text{O}$ occurs during the late Eocene to earliest Oligocene (Zones P15/16–NP21). Previous results from other North Atlantic DSDP sites (400A and 398) have significantly lower $\delta^{18}\text{O}$ values of benthic foraminifera, some by as much as 2‰ (refs 1–3). We believe that these differences result from diagenetic alteration of the sediments in the deeper-buried Sites 400A and 398.

Previous reports of Pacific and Southern Ocean DSDP sites noted that a 1.0–2.0‰ enrichment of ^{18}O occurs from the middle Eocene to early Oligocene in both planktonic and benthic foraminifera and that ~1‰ of this change occurs near the Eocene–Oligocene boundary^{4–9}. Eocene to Oligocene $\delta^{18}\text{O}$ records from three DSDP locations depart from this general Pacific–Southern Ocean trend: (1) in the Philippine Sea (Site 292) the ^{18}O enrichment occurs only in benthic foraminifera⁹; (2) in the South Atlantic (Site 357), an isotopic enrichment occurs between the middle and late Eocene¹⁰; and (3) in the North Atlantic (Site 398) the magnitude of the isotopic increase is twice that noted in the Pacific and Southern Oceans and the enrichment occurs near the middle/late Eocene boundary^{1,2}.

The Eocene to Oligocene oxygen isotopic enrichment in the North Atlantic is poorly documented. Strong diagenetic effects

on isotopic composition were noted in sites drilled in the Bay of Biscay (Site 400A) (refs 1, 3, 11) and off Portugal (Site 398) (refs 1, 2, 12, 13). Eocene benthic foraminiferal $\delta^{18}\text{O}$ values are low and calculated palaeotemperatures are correspondingly high (~17 °C)^{1,2} relative to Pacific, Southern Ocean^{4–9}, and South Atlantic¹⁰ $\delta^{18}\text{O}$ records. Such low $\delta^{18}\text{O}$ values and high inferred palaeotemperatures may result from diagenetic effects. It is necessary to distinguish between altered isotopic signals and original unaltered signals to determine abyssal palaeocirculation patterns.

We analysed the oxygen and carbon isotopic composition of early–middle Eocene to Oligocene benthic foraminifera at two North Atlantic DSDP sites (119 and 401). We chose these sites for the shallow burial depth of their Eocene sediments (~100–400 m) versus Sites 400A and 398 (~550–600 m). Late Eocene sediments are missing from Site 119. However, drilling at nearby Site 401 recovered a relatively complete Eocene section, but no Oligocene section¹⁴. Site 119 was drilled in 4,447 m of water on Cantabria Seamount¹⁵. Using the backtracking

Table 1 Oxygen and carbon isotopic data for Sites 119 and 401

Sample	Zonal age (Myr)	Taxa	$\delta^{18}\text{O}$	$\delta^{13}\text{C}$
Site 119				
12-CC	NP25 (24.0)	<i>Cibicidoides</i>	1.15	0.39
13-2 146–149 cm	NP25 (25.7)	<i>Cibicidoides</i>	1.40	0.30
13-CC	NP25 (26.3)*	<i>Cibicidoides</i>	1.54	0.53
14-CC	P21, NP24 (28.7)	<i>Cibicidoides</i>	1.36	–0.03
15-CC	P20/21, NP24 (31.0)*	<i>Cibicidoides</i>	1.61	–0.01
		<i>Gyroidinoides</i>	1.52	–0.33
			1.24	–0.47
			1.21	–0.46
			1.72	–0.68
		<i>Catapsydrax</i>	0.81	–0.59
16-2 119–121 cm	NP23 (31.6)	<i>Cibicidoides</i>	1.27	0.06
16-CC	NP23, P20/21 (32.3)*	<i>Cibicidoides</i>	1.81	0.56
			1.78	0.42
		<i>Gyroidinoides</i>	2.26	–0.06
			2.17	–0.23
		<i>Catapsydrax</i>	1.12	0.52
17-5 146–149 cm and 17-CC	NP22	<i>Catapsydrax</i>	0.84	0.71
17-CC	NP22 (34.7)	<i>Cibicidoides</i>	1.59	0.75
		<i>Gyroidinoides</i>	2.05	0.53
18-2 144–147 cm	NP21/22 (35.7)	<i>Cibicidoides</i>	1.35	0.47
18-CC	NP21 (37.0)	<i>Cibicidoides</i>	1.96	1.28
			2.02	1.27
		<i>Gyroidinoides</i>	2.56	0.76
			2.34	0.46
19-CC	Lower NP15 (47.0)	<i>Cibicidoides</i>	0.14	0.38
20-3 top	NP15 (47.4)	<i>Cibicidoides</i>	0.42	0.44
20-CC	NP15 (47.6)	<i>Cibicidoides</i>	0.43	0.53
		<i>Gyroidinoides</i>	0.79	0.05
21-CC	NP13 (49.3)	<i>Cibicidoides</i>	0.73	0.51
		<i>Gyroidinoides</i>	0.62	0.03
Site 401				
2-1 8–10 cm	<i>G. cerrolazulensis</i> Zone, NP21 (ref. 22) (37.5)	<i>Cibicidoides</i>	1.26	0.89
3-1 15–21 cm	P15/16 (38.9)	<i>Cibicidoides</i>	0.64	0.67
4-CC	P14 (41.0)	<i>Cibicidoides</i>	0.86	0.55
5-3 93–97 cm	P13 (42.8)	<i>Cibicidoides</i>	0.22	0.14
6-3 76–80 cm	P12 (43.9)	<i>Cibicidoides</i>	0.62	0.53
7-3 76–80 cm	P12 (45.3)	<i>Cibicidoides</i>	0.20	0.73
8-3 44–48 cm	P11 (46.6)	<i>Cibicidoides</i>	0.43	0.65
9-3 96–100 cm	P10 (47.7)	<i>Cibicidoides</i>	0.45	0.63
10-3 98–102 cm	P10 (49.0)	<i>Cibicidoides</i>	0.42	0.49

Nannoplankton zonations for Site 119 after ref. 20 and M.-P. Aubry, personal communication. Foraminiferal zonations for Site 119 after ref. 19 and K.G.M. work in preparation and for Site 401 after ref. 14.

* Discrepancies resulting from differences between zonal age and interpolated ages (indicated in parentheses in Myr) obtained by assuming constant sedimentation rate.

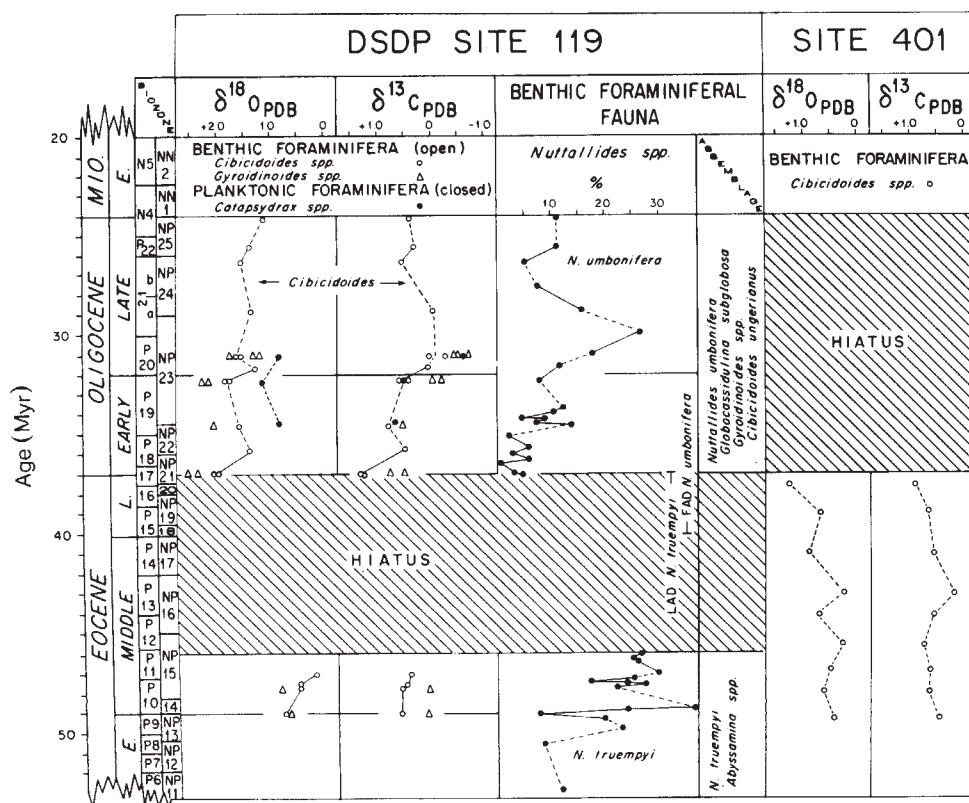


Fig. 1 Age versus foraminiferal isotopic composition for Sites 119 and 401 and faunal composition for Site 119. Last appearance datum (LAD) of *N. truempyi* and first appearance datum (FAD) of *N. umbonifera* after ref. 39.

method of Berger and Winterer¹⁶, we calculate that Site 119 was deeper than 3,000 m throughout the Eocene and Oligocene. Site 401 was drilled in the Bay of Biscay on the Armorican margin in 2,495 m of water¹⁷. Palaeobathymetric estimates place Site 401 near its present depth throughout the early Tertiary¹⁸. Biostratigraphic age control for the sites is taken from the *Initial Reports of the DSDP* Legs 12 (refs 15, 19–21) and 48 (refs 14, 17, 22); a biostratigraphic review and detailed palaeobathymetric estimates will be presented elsewhere (K.G.M., work in preparation).

Isotopic analyses were performed on mixed species of the genus *Cibicidoides*. Interregional isotopic variations of this taxon in Holocene core tops reflect the distribution of temperature, $\delta^{18}\text{O}$ of seawater, and $\delta^{13}\text{C}$ of ΣCO_2 in the modern

ocean^{23–26}. Although *Cibicidoides* species apparently secrete their tests lower in $\delta^{18}\text{O}$ than values calculated from the Epstein *et al.*²⁷ palaeotemperature equation, the offsets seem to be constant^{23–26}. In addition, we analysed paired samples of *Gyroidinoides* spp. and *Cibicidoides* spp. from Site 119; *Gyroidinoides* were $\sim 0.3\%$ enriched in ^{18}O relative to *Cibicidoides*. This offset is similar to differences observed between these taxa in studies of Holocene core-top sediments²⁶.

At Site 119, $\delta^{18}\text{O}$ values increase from $\sim 0.5\%$ in the early-middle Eocene (nannoplankton Zones NP13 to NP15) to $\sim 2.0\%$ in the earliest Oligocene (Zone NP21) (Fig. 1); $\delta^{13}\text{C}$ values correspondingly increase from ~ 0.4 to $\sim 1.3\%$ from the middle Eocene to earliest Oligocene. Unfortunately a 9-Myr hiatus precludes determination of the exact timing and nature of this isotopic change between the middle Eocene and earliest Oligocene in Site 119. In the Oligocene, $\delta^{18}\text{O}$ values decrease from ~ 2.0 to $\sim 1.1\%$. $\delta^{13}\text{C}$ values decrease to a minimum of $\sim 0.0\%$ in the middle Oligocene (Zones P20–P21), then increase again to $\sim 0.5\%$. At Site 401 (Fig. 1), $\delta^{18}\text{O}$ values are $\sim 0.5\%$ in the early-middle Eocene, similar to observed values in Site 119. $\delta^{18}\text{O}$ values increase $\sim 0.4\%$ in the late middle Eocene (Zone P14). A $\delta^{18}\text{O}$ increase of 0.6% occurs within the late Eocene (Zones P15/16–P17).

Combining the isotopic record of both sites (Fig. 2) suggests that a $\delta^{18}\text{O}$ increase of $\sim 1.4\%$ occurs from the late Eocene to earliest Oligocene in the North Atlantic. This change occurred within an interval that may be < 1 Myr (that is within Zone NP21) or as much as 4 Myr (between Zones P15 and NP21; see biostratigraphic zonations Table 1). There are pitfalls in combining sites of different palaeodepths to obtain a complete record; however, $\delta^{18}\text{O}$ values obtained for Sites 119 and 401 are similar in the overlapping section (middle Eocene). The modern temperature difference between these water depths in the Bay of Biscay is $< 1^\circ\text{C}$ (ref. 28), equivalent to an oxygen isotopic difference of $< 0.25\%$. These reasons justify combining the $\delta^{18}\text{O}$ records of Sites 119 and 401. Although the middle to late Eocene record may be found entirely within Site 401, the nature and timing of the $\delta^{18}\text{O}$ shift near the Eocene–Oligocene boundary involves comparing records from two different sites, and should be considered as preliminary. The Eocene–Oligocene interval in the North Atlantic is usually

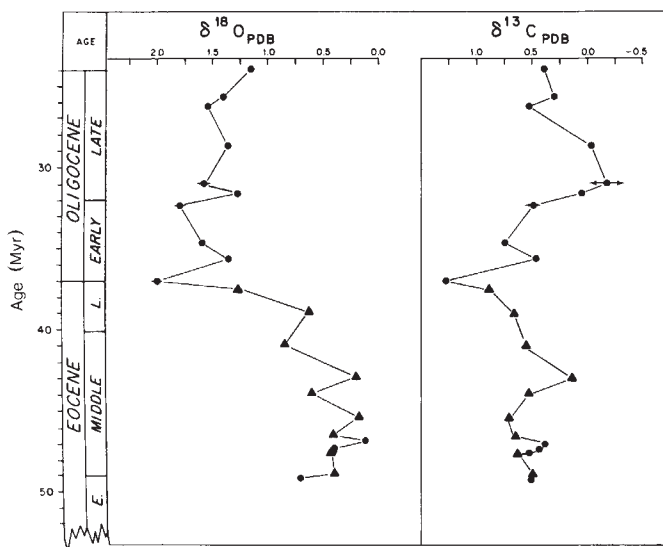


Fig. 2 Combined oxygen and carbon isotopic record obtained for *Cibicidoides* from Sites 119 (●) and 401 (▲). Where more than one value was obtained per sample, the mean value was plotted and the range indicated with arrows.

discontinuous^{29,30}, and a complete Eocene to Oligocene isotopic record from one site is not available at present.

The $\delta^{18}\text{O}$ records of Sites 119 and 401 apparently correlate with isotopic records from the Pacific and Southern Oceans⁴⁻⁸ where an ^{18}O enrichment occurs in the late Eocene to early Oligocene. In detail, the curves may not correlate. Keigwin⁹ showed that the ^{18}O enrichment occurs in the Pacific above the Eocene–Oligocene boundary, while in our record the change occurs from the late Eocene to earliest Oligocene. However, given the errors of the biostratigraphic age assignments, we consider that the enrichments may be synchronous. Further biostratigraphic studies are needed to determine if the enrichments are diachronous or synchronous.

Although the $\delta^{18}\text{O}$ values we report are similar to values obtained from sites in the Pacific and Southern Oceans⁴⁻⁹, they are markedly higher than Eocene $\delta^{18}\text{O}$ values obtained from other sites in the North Atlantic¹⁻³ (Fig. 3). In the Eocene, benthic foraminiferal $\delta^{18}\text{O}$ in Sites 400A and 398 are as much as 2‰ lower than in Sites 119 and 401 (Fig. 3). Such implied differences in bottom water temperature (8 °C) between sites of similar palaeodepth (for example, Sites 400A and 119) within the small basin of the Bay of Biscay are oceanographically unreasonable. As the total range of $\delta^{18}\text{O}$ values for benthic foraminifera within any Recent core-top sample²³⁻²⁶ or Palaeogene sample¹⁰ is <1‰, the 2‰ difference probably cannot result from sampling biases caused by selective dissolution or analysis of mixed benthic foraminiferal assemblages. However, the low values $\delta^{18}\text{O}$ observed can result from diagenetic alteration of the foraminiferal test at high temperatures associated with great burial depth^{2,13}. While simple addition of calcite overgrowths of the test would be sufficient to lower $\delta^{18}\text{O}$ values, recrystallization of the foraminiferal calcite is necessary to produce both the similar $\delta^{18}\text{O}$ values of planktonic and benthic foraminifera in the Eocene of Sites 400A and 398 and the high bottom water temperatures (~17 °C) calculated for these sites¹⁻³. At Site 401, $\delta^{18}\text{O}$ of planktonic foraminifera are substantially lower than benthic foraminifera³, supporting the idea that this site is not substantially affected by diagenetic alteration. Similarly, planktonic foraminifera have substantially lower $\delta^{18}\text{O}$ composition than benthic foraminifera in the Oligocene of Site 119; due to dissolution, sufficient planktonic

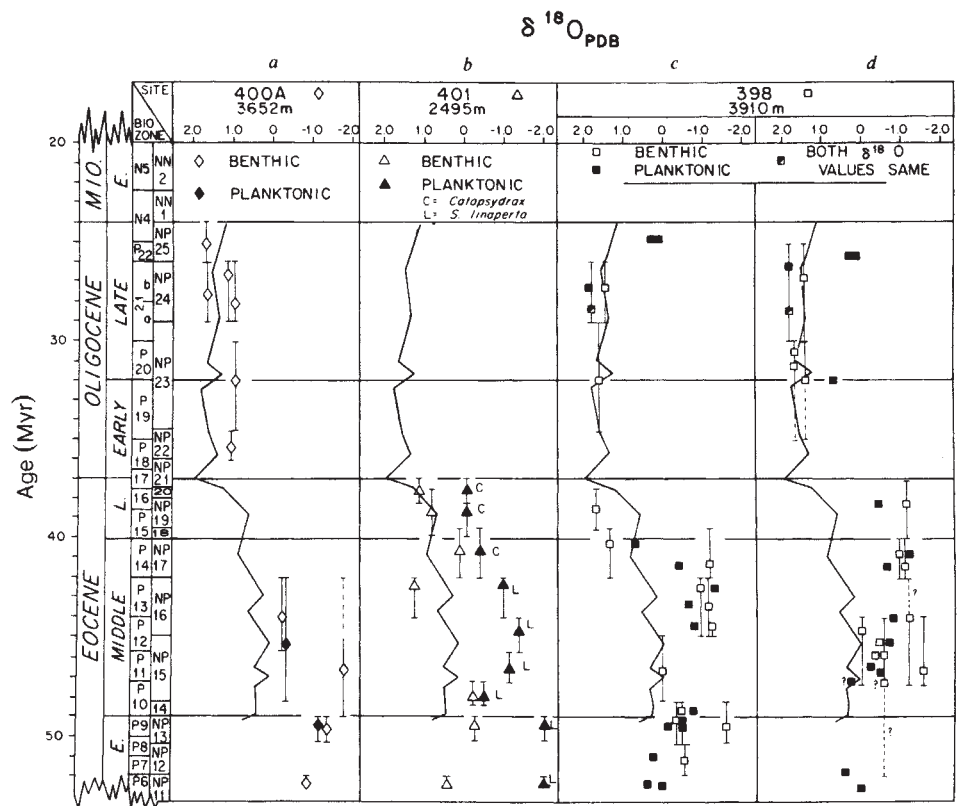
foraminifera were not available for isotopic analyses of the Eocene of Site 119.

Limits can be calculated for the abyssal temperature change in the Bay of Biscay. Shackleton and Kennett⁶ estimated that $\delta^{18}\text{O}$ of seawater before the formation of the present ice sheets was -1.2‰ (PDB). Assuming that *Cibicides* precipitates CaCO_3 0.65‰ lower than equilibrium²⁶ and that there was no significant glacial ice buildup, the increase in benthic foraminiferal $\delta^{18}\text{O}$ from Sites 119 and 401 corresponds to a maximum decrease of bottom-water temperatures from 8 to 2 °C between the middle Eocene and early Oligocene. Alternatively, assuming glacial buildup equivalent to the present ice sheets, the increase represents a minimum change in bottom-water temperature from 8 to 6 °C.

The benthic temperature drop in the late Eocene to early Oligocene in the Pacific and Southern Oceans has been attributed to the first formation of bottom waters in the Antarctic region⁷; however, the North Atlantic basins may have had a different source of cold bottom water. Seismic stratigraphical evidence from the northern North Atlantic Ocean indicates that northern sources of vigorously circulating bottom water began in the late Eocene to early Oligocene³⁰⁻³². Miller and Tucholke³⁰ suggested that this bottom water was of Arctic origin and that it flowed through the Norwegian–Greenland Sea, the Faeroe–Shetland Channel, and possibly through the Denmark Straits into the North Atlantic.

The isotopic changes observed in Site 119 correlate with a change from a benthic foraminiferal assemblage dominated by *Nuttallides truempyi* to an assemblage dominated by *N. umbonifera* (Fig. 1); however, the hiatus prevents an exact determination of the timing of this faunal change. Within the Oligocene, *N. umbonifera* reaches a peak in abundance correlating with a $\delta^{13}\text{C}$ minimum. As *N. umbonifera* is negatively correlated with carbonate saturation in the modern ocean³³ and lower $\delta^{13}\text{C}$ values are often associated with older water masses³⁴⁻³⁶, we speculate that the isotopic and faunal records of Site 119 reflect a change from younger, less corrosive water in the early Oligocene to older, more corrosive bottom water in the middle Oligocene. The change from lower carbon and oxygen values in the Eocene to higher carbon and oxygen values in the earliest Oligocene may reflect a change from older and

Fig. 3 Comparison of previously reported¹ Eocene to Oligocene $\delta^{18}\text{O}$ values for benthic and planktonic foraminifera from North Atlantic DSDP sites. Combined oxygen isotopic curve obtained from Sites 119 and 401 is superimposed on each (solid line). Columns *a* and *b* are from Bay of Biscay Sites 400A and 401, respectively. Columns *c* and *d* are from Site 398 off Portugal: *c* represents ages assigned using calcareous nannoplankton³⁷, *d* represents the same data with ages assigned using planktonic foraminifera³⁸. Biostratigraphic ages for Sites 400A and 401 have been assigned using planktonic foraminifera¹⁴ and nannoplankton²². Error bars indicate length of biostratigraphic zone(s) to which the sample has been assigned; they have been omitted for clarity from planktonic foraminiferal $\delta^{18}\text{O}$ values in columns *c* and *d*.



warmer Eocene bottom water to colder, younger, more vigorously circulating bottom waters of northern origin in the early Oligocene.

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Compound eyes project stripes on the optic tectum in *Xenopus*

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In the vertebrate nervous system it is commonly found that when two similar nervous structures innervate a third, each innervating structure colonizes distinct areas of the target in a striped manner. Much of our knowledge of the rules of neuronal connectivity derives from the study of the development of the frog visual system, which has the advantages of being simple, and accessible throughout embryonic development. When two separate eyes are made to innervate a single frog optic tectum, the two projections separate into stripes, reminiscent of the ocular dominance stripes of the mammalian visual cortex¹. We report here an experiment in which the projection from a single eye, surgically constructed in the embryo out of two matching half-eyes (a 'compound' eye), separates out into stripes. The observation that a single eye can produce a striped projection rules out many hypotheses of how stripes are formed, and provides evidence that the formation of an ordered projection on the optic tectum requires the operation of two separate mechanisms.

In lower vertebrates, for which much is known of the mechanisms of neural connectivity^{2,3}, the retinotectal projection is completely crossed, and direct binocular innervation occurs by a secondary pathway⁴. Experimental manipulation is therefore needed to induce two retinæ to innervate the same target. It is then found that the tectum is divided up between the fibres from the two eyes, in some cases to form eye-specific patches, and in others stripes⁵⁻⁹. In order for stripes to form, there must be some means of distinguishing the fibres from one retina from those from the other; three possible mechanisms come to mind. (1) Fibres carry a record of their side of origin, which enables those from a left eye to be distinguished from those from a right eye. The same effect would be produced if the positional information in the eye were carried by a varying signal such as a biological oscillator¹⁰. The two eyes could therefore be out of phase. (2) Terminal arbors are only stable on their target if neighbouring arbors are from neighbouring ganglion cells. (3) The mechanics of fibre growth are such that the fibres from two separate eyes remain segregated through to their site of termination.

There are various ways of producing double innervation of the visual centres in lower vertebrates, but to determine which of the above theories is correct, a useful technique is surgically to place together in the same eyecup of an embryonic *Xenopus*, two half-eye rudiments of matching retinal origin (for example, two nasal halves) to create a compound eye¹¹. This results in a retinotectal map in which each of the two half-eyes projects across the entire tectum, superimposed on each other, and with corresponding retinal positions of origin mapping to the same area of tectum. In *Xenopus* the operation is done before any nerve fibres have grown out of the eye, and there is therefore minimal disruption of the normal development of the visual system; the fibres follow the normal pathway to the brain. This also allows the full development of the projection to be studied, and its topography can be assessed electrophysiologically, so that the relationship between the mechanisms of map-making and stripe formation can be studied.

Compound eyes were made by standard procedures¹¹. One half of the eye rudiment of a stage 29-31 *Xenopus* embryo was replaced with the opposite half from another embryo of the same age, so that the eye had two nasal halves (NN), two ventral halves (VV) or two temporal halves (TT). The donor half was from the opposite side of the head. Three of the animals also had the other eye removed. The animals were reared for 1-2 months after metamorphosis, then the projections made by the compound eyes were assessed.

The success of the compound eye operations was confirmed by electrophysiological mapping. The retina was then lesioned near the optic nerve head so as to cut most of the fibres from either the host or donor half, and a ~20% solution of horseradish peroxidase (HRP) was then injected over the lesion. After 48 h, the animal was anaesthetized, then perfused and fixed in 2.5% glutaraldehyde in 0.1 M phosphate buffer. The brain was removed and processed using the method of Adams¹² with the following modifications: incubation was in 1% CoCl₂ for 1 h, then in diaminobenzidine acid solution for 90 min, followed by the addition of 0.3% H₂O₂ (4 ml per 100 ml) at 0 °C for 20 min. The brains were cleared in methyl salicylate, and examined as whole mounts. A total of 11 animals were examined, of which 9 had both good maps and HRP fills.

Electrophysiological mapping was carried out using standard techniques. In all cases the maps obtained were of the classical compound-eye type^{11,13}. Most of the recording positions produced responses that could be driven by two distinct areas of the visual field. These areas were so arranged that (1) each of the constituent halves of the compound eye projected over the entire tectum in such a way that areas of retina of similar origin projected to the same area of tectum, and (2) the orientation of each map was that predicted if the appropriate half-eye were part of a normal eye.

In all cases the neuropil was labelled discontinuously. The neuropil was fairly superficial, and fibres of passage were seen

at the same depth and deeper than it. In eight of the nine cases the tectum was striped. Typically there were four or five labelled areas. As measured in the cleared preparations, the stripes were of constant width (60–80 μm) and there was a constant inter-stripe distance of 60–80 μm . The stripes ran rostrocaudally in all cases, and were usually straight, or sometimes slightly wavy. In some cases the stripes coalesced at one extremity of the tectum. This could have been due either to the HRP leaking into the half-eye that it was not intended to fill, or to the mechanism of stripe formation (see below). The actual part of the tectum that was covered depended on the class of compound eye, since the area immediately surrounding the optic nerve head projects to different tectal areas in different classes of compound eye, and it was not possible to fill the chosen half-eye right up to the optic nerve head. Thus in the NN preparations there was no filling of neuropil at the front of the tectum; the midline of the NN eye projected to the rostral tectum. It made no difference to the result whether the host or donor half of the eye was filled.

Optic fibres followed the normal route to the brain, and the manner of their delivery to the tectum was characteristic of the pathways of the various types of compound eye (refs 14, 15 and J.W.F. and R. M. Gaze, in preparation). The only structure that showed striping was the neuropil; the fibres running to the areas of neuropil formed an even sheet over the tectal surface, with no evidence of being divided into separate bands each heading to a specific stripe. No stripes were observed in the optic tract. In addition, fibres of passage were seen between the stripes. In the NN preparations, these fibres ran rostrocaudally, as did the stripes (Fig. 1). In the case of the VVs, however, the fibres ran across the tectum in a rostro-medial to caudo-lateral direction, having all arrived at the tectum by the medial brachium of the tract. The stripes, however, still ran rostrocaudally (Fig. 2). The pretectal nucleus, neuropil of Bellonci and basal optic nucleus, where filled, showed no striping.

In our preparations the fibres from the two half-retinae making up the compound eye travel in a single optic nerve along a normal pathway to the optic tectum. Stripes are visible only in their terminal arborizations. This rules out any mechanism relying on fibres maintaining contact with their original neighbours throughout the optic pathway; nor can stripes be due to the fibres from one eye taking an abnormal pathway, as could be the case in 'three-eyed' frogs⁷. Our experiment also rules out the possibility of stripes being due to an oscillating positional signal.

The two possible mechanisms remaining, therefore, are that either left and right eyes carry specific labels, or that there is a mechanism which stabilizes arbors that are neighbours on the



Fig. 2 Camera lucida drawing of a whole mount of a tectum innervated by a double ventral eye. Orientation as in Fig. 1. The stripes again run rostrocaudally, but the fibres to them run from rostro-medial to caudo-lateral tectum.

tectum, and whose retinal ganglion cells are also neighbours. The data of Law and Constantine-Paton⁹ make the first possibility unlikely, but we are testing this by constructing compound eyes in which both halves are from the same side of the head. The second mechanism would result in stripes coalescing in the region to which the retinal midline projects; our results provide some evidence for this.

The neurophysiological and neuroanatomical evidence strongly suggests that the mechanism responsible for the orientation and internal ordering of the retinotectal map in amphibia depends on a set of labels encoding retinal position^{2,3,16}. This allows, for example, fibres from two originally nasal poles of a double nasal eye to project to the same area of tectum. The presence of stripes, however, seems a direct contradiction of this; the two sets of fibres are directed to the same area of tectum only to be separated again. It seems probable, therefore, that the retinotectal map forms in two stages: first the ingrowing optic fibres are guided to terminate on an area of tectum which is determined by the positional labels carried by the retinal ganglion cells from which they came. Once on the tectum, the fibres produce terminal arborizations which interact according to a different set of rules, as discussed above; in doubly innervated tecta this results in the formation of stripes. This is consistent with the suggestion that stripes develop from an initially diffuse projection⁸. It would certainly require less rearrangement of fibres to produce stripes from a retinotopic map than retinotopy from a striped but disordered one.

In normal development, we believe that the map orientation and a rough degree of retinotopic order are defined by the pathways which fibres follow in growing on to the tectum. Interactions between neighbouring arbors of the type that lead to stripe formation on doubly innervated tecta refine the map and allow it to accommodate to the arrival of new fibres and the growth of the tectum. These two mechanisms correspond to the division suggested by Law and Constantine-Paton⁸.

The stripes in our preparations always ran rostrocaudally. This orientation is clearly not determined by the direction of ingrowth of the optic fibres, as when this is altered (as in the VV animals), the stripes still run rostrocaudally. The most obvious explanation for the direction is that it reflects the mode of growth of the tectum, which grows from its caudal margin¹⁷. Thus, patches of fibres from the two half-eyes formed when the tectum is very small might be expected to extend caudally as the tectum grows. In *Xenopus*, when two optic nerves are induced to innervate a fully grown tectum by regeneration (ref.

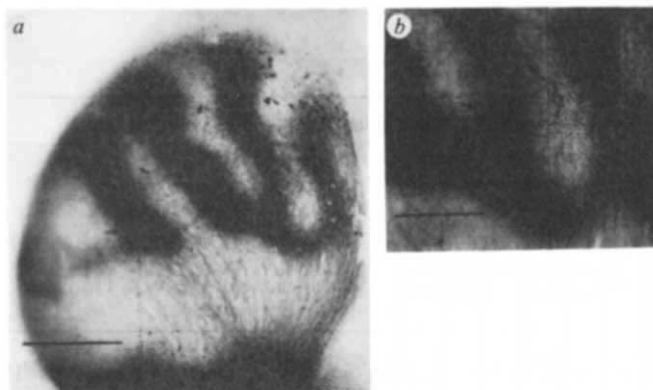


Fig. 1 *a*, Dorsal view of a whole mount of a tectum innervated by a double nasal eye: medial tectum to the right, caudal to the top. The donor half of the eye was filled with HRP. Stripes run rostrocaudally; the fibres running to them are unstriped. Scale bar, 200 μm . *b*, Detail from *a*. Fibres of passage can be seen running between and below the stripes. Scale bar, 100 μm .

6 and D.J.W. and R. M. Gaze, in preparation), the regions of tectum innervated by one eye are not stripes but patches, with no discernible orientation. This may reflect the fact that the tectum is already fully grown and the initial regenerated projection is disorganized¹⁸. It is interesting that the constant width of the stripes corresponds approximately to the size of the optic fibre arborizations on the *Xenopus* optic tectum^{19,20}.

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Adhesion-dependent heparin production by platelets

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It is well known that platelets are involved in blood clotting. In addition, substances of the platelet granules or on the platelet surface cooperate with plasma and vessel wall components to modulate control of haemostasis¹. Here we provide evidence that platelets adhered to a substrate can release heparin.

When fresh human platelets were isolated from plasma and incubated *in vitro* in suspension or allowed to adhere to a solid surface, the medium of adherent cells showed an anticoagulant activity that was absent in the medium of suspended cells. As shown in Table 1, the production of the activity is adhesion dependent but does not require a specific adhesion substrate; however, artificial rafts containing compounds found in the subendothelial basal lamina were more effective than plastic or glass surfaces in inducing the release of the anticoagulant activity. This activity is resistant to heat, proteolysis, hyaluronidase and chondroitinase, is sensitive to nitrous acid, retained by anionic exchangers and eluted by cationic exchangers.

Figure 1a shows the electrophoretic analysis of platelet glycosaminoglycans. Chondroitin 4-sulphate is the only glycosaminoglycan present in suspended platelets, with sialosaccharides and trace amounts of dermatan sulphate. In contrast, in adhesion conditions, a complex pattern of glycosaminoglycans is present, the bands being identified as chondroitin 4-sulphate, sialosaccharides, dermatan and heparan sulphates

Table 1 Anticoagulant activity of media from platelets incubated in different conditions and sensitivity to various degradative agents

Culture conditions	Thrombin time (s)
No platelet	20/11
Suspended platelets	
Platelets adherent to glass, plastic or polyvinylchloride	120/150
Platelets adherent to artificial rafts containing type IV collagen, fibronectin and laminin	160/200
Type of degradation or treatment on glass-adherent platelet medium	
Exhaustive proteolysis, chondroitinase ABC, testicular hyaluronidase, heat (10 min at 100 °C) passage through AG 50 X8	120/160
Nitrous acid, passage through AG 1 X2	30/40

Platelets were isolated from pooled human blood containing either EDTA (0.1%) or sodium citrate (0.38%) as anticoagulant. The blood was allowed to sediment spontaneously at room temperature and the upper phase was centrifuged at low speed (10g for 10 min). The supernatant was centrifuged at 1,000g for 2 min to sediment residual leukocytes and the platelets were then sedimented from the second supernatant by prolonged low-speed centrifugation (30 min at 160g). Leukocyte contamination was less than 1% as checked with the light microscope after May-Grunwald-Giemsa staining. Platelets were suspended in Hank's saline containing 1 mg ml⁻¹ glucose; the concentration was 3 × 10⁸ cells per ml for the suspension experiments and 10-fold diluted for adhesion experiments. Cells were layered on a solid surface with a ratio of 3 × 10⁶ cells per cm² and allowed to adhere to the substrate for 15 min. At the end of this incubation up to 60% of the cells were adherent. The medium was collected and centrifuged to remove non-adherent platelets. Artificial collagen rafts containing basement membrane non-collagenous proteins were prepared according to Rubin *et al.*³. All the matrix compounds were a gift of Dr R. Timpl. The media were concentrated 40-fold by ultrafiltration on Amicon apparatus equipped with a YM5 membrane and used directly for thrombin time assay. The test was carried out as follows: 100 µl of plasma pool were incubated at 37 °C with an equal volume of the tested sample, 100 µl of a thrombin solution (Boehringer-Mannheim) were added and the coagulation time was measured. Alternatively, media were digested with papain (0.1 mg ml⁻¹) at 60 °C for 48 h in the presence of 5 mM β-mercaptoethanol at neutral pH. After heating for 1 h at 95 °C to inactivate the protease, various aliquots were treated with hyaluronidase, chondroitinase or nitrous acid as described elsewhere⁴. After dialysis and concentration to the original volume, the processed samples were again assayed for anticoagulant activity by the same test. Aliquots were passed through cationic or anionic exchangers (Bio-Rad), neutralized, dialysed, concentrated to the original volume and tested for the anticoagulant activity.

Table 2 Pulse labelling of platelet glycosaminoglycans

Compound	C4S	HS/DS	HP
Densitometric value of the electrophoretic band (A units)	0.37	0.15	0.32
³ H-glucosamine (c.p.m.)	9	134	313
³⁵ S-inorganic sulphate (c.p.m.)	7	49	127

Adherent platelet medium was added with 1 µCi ml⁻¹ of ³H-glucosamine and 5 µCi ml⁻¹ ³⁵S-inorganic sulphate (NEN) and the cells were incubated for 30 min. Glycosaminoglycans were isolated as described in Fig. 1 legend, subjected to electrophoresis at pH 5.0 and densitometrically recorded. The cellulose acetate was cut into strips corresponding to the well resolved bands of chondroitin 4-sulphate (C4S) and heparin (HP) and the less resolved bands of heparan and dermatan sulphates (HS/DS). The acetate strips were then counted in a Bray's scintillation mixture.

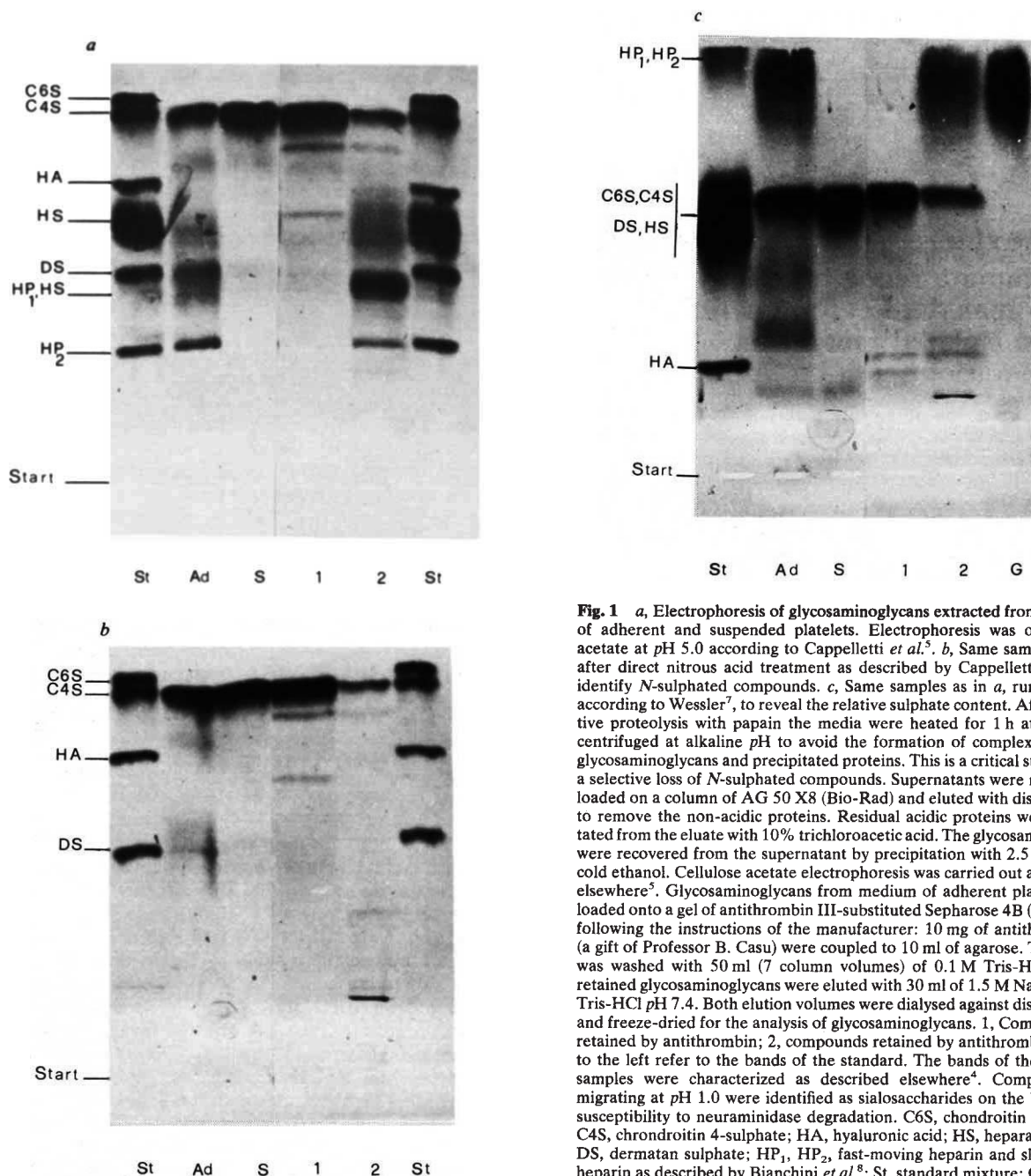


Fig. 1 *a*, Electrophoresis of glycosaminoglycans extracted from the media of adherent and suspended platelets. Electrophoresis was on cellulose acetate at pH 5.0 according to Cappelletti *et al.*⁵. *b*, Same samples as in *a* after direct nitrous acid treatment as described by Cappelletti *et al.*⁶, to identify *N*-sulphated compounds. *c*, Same samples as in *a*, run at pH 1.0 according to Wessler⁷, to reveal the relative sulphate content. After exhaustive proteolysis with papain the media were heated for 1 h at 95°C and centrifuged at alkaline pH to avoid the formation of complexes between glycosaminoglycans and precipitated proteins. This is a critical step to avoid a selective loss of *N*-sulphated compounds. Supernatants were neutralized, loaded on a column of AG 50 X8 (Bio-Rad) and eluted with distilled water to remove the non-acidic proteins. Residual acidic proteins were precipitated from the eluate with 10% trichloroacetic acid. The glycosaminoglycans were recovered from the supernatant by precipitation with 2.5 volumes of cold ethanol. Cellulose acetate electrophoresis was carried out as described elsewhere⁵. Glycosaminoglycans from medium of adherent platelets were loaded onto a gel of antithrombin III-substituted Sepharose 4B (Pharmacia) following the instructions of the manufacturer: 10 mg of antithrombin III (a gift of Professor B. Casu) were coupled to 10 ml of agarose. The column was washed with 50 ml (7 column volumes) of 0.1 M Tris-HCl pH 7.4; retained glycosaminoglycans were eluted with 30 ml of 1.5 M NaCl in 0.1 M Tris-HCl pH 7.4. Both elution volumes were dialysed against distilled water and freeze-dried for the analysis of glycosaminoglycans. 1, Compounds not retained by antithrombin; 2, compounds retained by antithrombin. Letters to the left refer to the bands of the standard. The bands of the biological samples were characterized as described elsewhere⁴. Compounds not migrating at pH 1.0 were identified as sialosaccharides on the basis of the susceptibility to neuraminidase degradation. C6S, chondroitin 6-sulphate; C4S, chondroitin 4-sulphate; HA, hyaluronic acid; HS, heparan sulphate; DS, dermatan sulphate; HP₁, HP₂, fast-moving heparin and slow-moving heparin as described by Bianchini *et al.*⁸; St, standard mixture; G, standard heparin (Glaxo).

and heparin. Figure 1 also shows that the compounds of adherent platelets exhibit a differential affinity to antithrombin III: heparan sulphate and heparin bind to antithrombin while others do not.

Figure 1*b,c* shows the ability of nitrous acid to destroy heparan sulphate and heparin as well as the high sulphate content of heparin. Commercial and platelet heparin was diluted to obtain solutions producing the same slowing effect on the thrombin time assay and the same volume of these solutions was then run on electrophoresis, stained and recorded by densitometry. A comparison of the peak areas showed anticoagulant activity of platelet heparin to be higher than that of the commercial standards.

When adherent cells were pulse labelled with radioactive precursors (see Table 2), chondroitin 4-sulphate took up almost no label whereas the other compounds, particularly heparin, were labelled by both radioactive glucosamine and sulphate, suggesting synthesis or a post-synthetic processing of *N*-sulphated glycosaminoglycans.

Platelets are known to release several clotting factors on aggregation, including fibrinogen, thrombin, coagulation factor V and the anti-heparin platelet factor IV². The present finding provides evidence of heparin release by a major blood cell subclass, thus indicating a possible source of endogenous heparin. Furthermore, it may indicate an alternative, non-thrombogenic role for platelets in vessel wall repair and control of clotting.

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Inhibition of dopamine biosynthesis by gonadotropin-releasing hormone in rat

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There is evidence that the neuroendocrine system can be modulated by endogenous opioid peptides (for a review see ref. 1). Recently Rotsztein *et al.*² have ruled out a direct effect of Met-enkephalin on release of gonadotropin-releasing hormone (GnRH). Instead they postulated that Met-enkephalin inhibits the secretion of dopamine from dopaminergic neurones thereby reducing the dopamine-stimulated release of GnRH from the hypothalamus. We report here evidence that GnRH can itself suppress dopamine synthesis in the rat. The fact that the dopamine neurone and GnRH-secreting cell are adjacent to each other³ suggests a feedback mechanism of regulating GnRH release.

Sprague-Dawley rats (180–230 g) were killed by decapitation. The corpus striatum was dissected on ice and homogenized in 10 vols 0.32 M sucrose using a Teflon pestle tissue homogenizer. After centrifugation at 1,000g for 15 min, 50 μ l aliquots of the synaptosome-containing supernatant were incubated with 150 μ l of physiological medium containing (mM): 125 NaCl; 1.48 CaCl₂; 4.8 KCl; 2.5 MgSO₄; 22 NaH₂PO₄; 10 NaHCO₃; 16 glucose, to give a final pH of 6.6 after equilibration with 95% O₂–5% CO₂ at 37°C. [1-¹⁴C]tyrosine (specific activity 50 mCi mmol⁻¹) at a concentration of 10 μ M was added to the preparation, together with bacitracin (10⁻⁵ M) to reduce the degradation of polypeptides. GnRH, Met-enkephalin or naloxone were added to the incubation medium with 10 μ l 0.1 M phosphorous buffer pH 6.6 as carrier; controls received the buffer alone. The method used to determine dopamine synthesis was modified from that of Weiner⁴ and Kuczenski⁵, who measured the release of ¹⁴CO₂ from [1-¹⁴C]tyrosine. We simplified the incubation medium to mimic the cerebrospinal fluid and used a respirometer to supply oxygen and allow continuous measurement of the ¹⁴CO₂ output from the tissue⁶. Addition of iodotyrosine (5 $\times$ 10⁻⁴ M) to the incubation medium inhibits >90% of the ¹⁴CO₂ release⁷.

Dopamine formation was calculated when the ¹⁴CO₂ output had reached a steady state, that is, after 50 min incubation. The rate of dopamine synthesis in 10 preparations was 0.2 $\pm$ 0.02 mol mg⁻¹ min⁻¹ (mean $\pm$ s.d.).

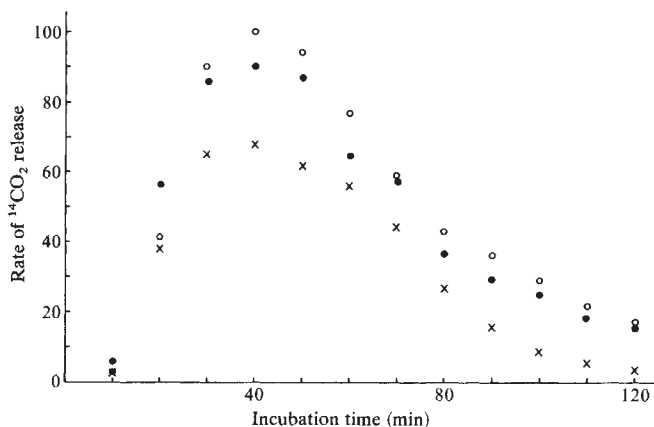


Fig. 1 Rate of ¹⁴CO₂ release from labelled tyrosine plotted against incubation time. The exponential increase in rate before 50 min of incubation is from isotopic equilibrium⁶. The rate of ¹⁴CO₂ release maintained steady-state values between 50 and 120 min of incubation. GnRH (5 $\times$ 10⁻⁶ M) added to the preparation (×) gave ~30% inhibition of dopamine synthesis. Naloxone at 5 $\times$ 10⁻⁶ M together with GnRH blocked 70% of this inhibition (●). ○, Control.

Met-enkephalin (10⁻⁵ M) added to the incubation mixture reduced ¹⁴CO₂ release by ~40%. Although some of this inhibition could be due to the degradation of the enkephalin releasing unlabelled tyrosine, we showed by kinetic studies using added unlabelled tyrosine that >10% of this inhibition was a direct effect. Other opioid δ -receptor drugs, for example, D-Ala²-Met-enkephalin and Metkephamid⁸ at 10⁻⁵ M also inhibited dopamine synthesis by ~10% and 20% respectively and this inhibition was not blocked by naloxone. In contrast, the 11% inhibition of dopamine synthesis by sufentanyl¹ (10⁻⁵ M), a μ -receptor drug, was almost totally blocked by naloxone.

When GnRH (5 $\times$ 10⁻⁶ M) was incubated with the synaptosomal preparation, the ¹⁴CO₂ release was reduced to 73.4 $\pm$ 6.6% of the normal rate. (The relative amounts of ¹⁴CO₂ released from the added tyrosine are shown in Fig. 1.) Naloxone (5 $\times$ 10⁻⁶ M) together with GnRH (5 $\times$ 10⁻⁶ M) blocked ~70% of the inhibitory effect. The normal ¹⁴CO₂ release was reduced by GnRH at 1 $\times$ 10⁻⁶ M and 5 $\times$ 10⁻⁷ M to 83.1 $\pm$ 3.8% and 89.9 $\pm$ 2.7% respectively. GnRH at a concentration of <1 $\times$ 10⁻⁸ M had no significant effect.

These data show that GnRH and δ - and μ -receptor opioids all have similar inhibitory effects on dopamine synthesis. We therefore postulate that GnRH exerts a negative feedback action on dopaminergic neurones, that is, GnRH inhibits its own release by inhibiting dopamine synthesis.

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Glutamate regulates adenylate cyclase and guanylate cyclase activities in an isolated membrane preparation from insect muscle

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It is generally believed that glutamate serves as a neurotransmitter at many vertebrate and invertebrate synapses. There is, however, little information concerning the possible involvement of cyclic nucleotides as intracellular second messengers for any of the postsynaptic actions of glutamate. Direct activation of adenylate cyclase has been reported for several neurotransmitters and neuromodulators¹, but no such effect has been reported for glutamate. Moreover, although several neurotransmitters including glutamate have been shown to increase cyclic GMP levels in intact preparations of nerve and muscle tissue^{2,3} apparently by promoting Ca²⁺ entry into the cells, no neurotransmitter has been shown to activate guanylate cyclase in a cell-free preparation. L-Glutamate is the prime candidate for the neurotransmitter at the excitatory neuromuscular synapses in insects⁴. Moreover, recent studies have shown that glutamate application raises both cyclic AMP⁵ and cyclic GMP levels (P.M.C., unpublished observations) in insect muscle. To determine the mechanism underlying these increases, we have studied the effect of glutamate in a membrane fraction prepared from this muscle, and report here that the neurotransmitter activates both adenylate cyclase and guanylate cyclase in the membrane preparation, and that the activation by glutamate of guanylate cyclase is calcium independent.

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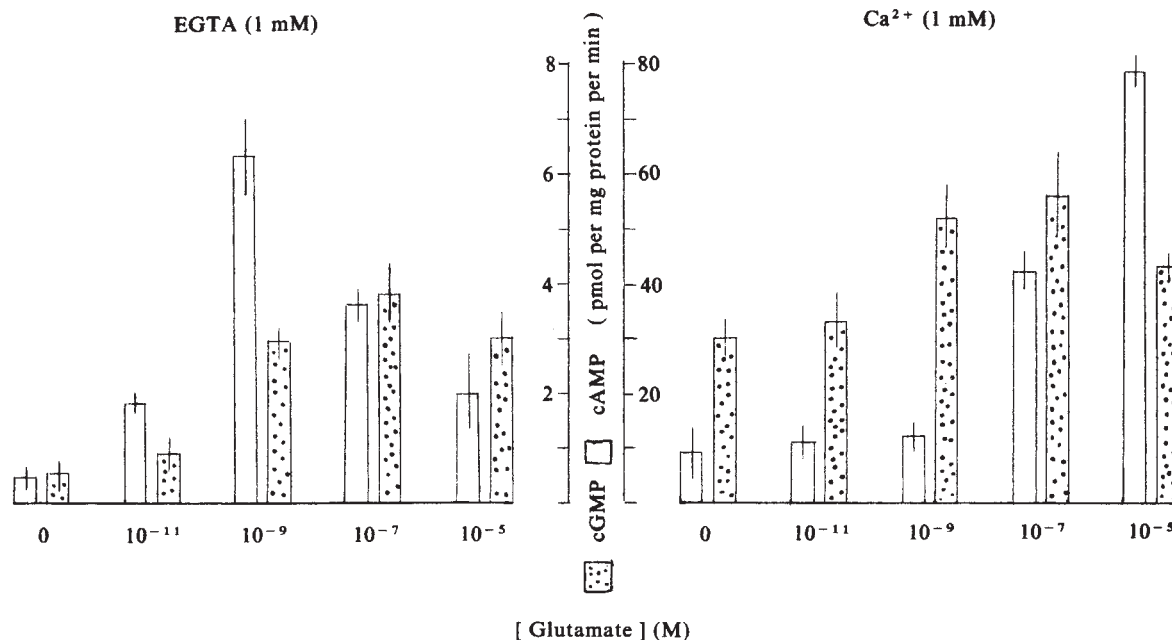


Fig. 1 Glutamate stimulation of cyclic AMP (cAMP) and cyclic GMP (cGMP) production by a membrane preparation from the body-wall muscles of the dipteran *Sarcophaga*. Third instar larvae of the insect were used. The central nervous system, digestive system and fat body were removed and the body-wall muscles were scraped from the cuticle and then homogenized on ice in isotonic saline (30 larvae per 25 ml) (NaCl, 120 mM; KCl, 2 mM; CaCl₂, 0.5 mM; thiourea, 1 mM; Tris-HCl, 10 mM, pH 6.8) in a Teflon/glass homogenizer (10 passes at 100 r.p.m.). The homogenate was centrifuged at 4,000g for 30 min and the supernatant was collected and centrifuged at 280,000g for 60 min. The supernatant (S2) was removed and the pellet (P2) lysed by resuspension in 25 ml of hypotonic saline (10 mM Tris, 1 mM thiourea, and either 0.5 mM CaCl₂ for 'calcium' incubations or 1 mM EGTA for calcium-free incubations). The suspension was then repelleted by centrifugation at 280,000g for 60 min. The pellet formed (P3) was resuspended in 2 ml cyclase assay buffer (10 mM NaCl, 10 mM Tris-HCl, 10 mM MgCl₂, 0.5 mM MnCl₂, 0.05 mM alanine, 1 mM thiourea and either 0.5 mM CaCl₂ or 1 mM EGTA, pH 6.8); adenylate and guanylate cyclase activities were assayed concurrently and the cyclase reaction started by the addition of an equal volume of cyclase assay buffer containing 2 mM ATP, 1 mM GTP and 2% albumin. Incubations were stopped by the addition of five vol of acidified alcohol (0.5 ml MHCl in 100 ml ethanol). Samples were evaporated to dryness at 55 °C under nitrogen and then extracted in extraction buffer (10 mM Tris, 1 mM EDTA pH 6.8). Cyclic AMP was assayed by the method of Brown *et al.*¹⁰. Cyclic GMP was assayed by radioimmunoassay as described elsewhere¹¹. The protein content of samples was determined using the Lowry folin-phenol method¹². Each point represents the mean $\pm$ s.e.m. of at least three estimations. In control experiments it was found that calcium was required during the initial homogenization to retain cyclase activity, whereas subsequent lysis of membrane fractions in calcium-free hypotonic saline did not result in loss of activity. Assays of marker enzymes carried out on the original homogenate, supernatant (S2) and resuspended cyclase preparation (P3) showed that the cyclase preparation contained the plasma membrane marker enzyme cholinesterase but was virtually free of the cytoplasmic enzyme lactate dehydrogenase and the mitochondrial enzyme glutamate dehydrogenase. Assays of adenylate cyclase and guanylate cyclase were carried out simultaneously to show that each enzyme was activated independently. This procedure is only possible for the particulate enzymes as the particulate form of guanylate cyclase is not markedly inhibited by ATP in the same way as the soluble form¹³.

Figure 1 shows the effect of various concentrations of glutamate on production of cyclic AMP and cyclic GMP by a membrane preparation of body-wall muscle from the dipteran *Sarcophaga*. Adenylate cyclase activity was stimulated in either the presence or absence of calcium. However, stimulation of cyclic AMP synthesis required a lower concentration of glutamate in the absence of calcium (activation occurring at a glutamate concentration $>10^{-11}$ M) than in the presence of calcium ($>10^{-9}$ M glutamate). In the absence of calcium, maximal stimulation of adenylate cyclase activity was observed at $\sim 10^{-9}$ M glutamate, inhibition occurring at higher concentrations of the neurotransmitter. Guanylate cyclase activity was also stimulated in either the presence or absence of calcium, maximal stimulation being observed at 0.1 μ M glutamate in both cases. The similarity of the dose-response curves as a function of glutamate concentration in the absence and presence of calcium (Fig. 2) indicate that this enzyme activity can be stimulated by either of two mechanisms, one of which is glutamate dependent and calcium independent, the other being calcium dependent and glutamate independent.

The fact that very low concentrations of glutamate are effective in stimulating adenylate cyclase and guanylate cyclase activities suggests that these intracellular messenger systems could be activated by the very low concentrations of transmitter released during spontaneous quantal discharge⁶.

Enzyme activity was proportional to incubation time for both guanylate cyclase (Fig. 3) and adenylate cyclase (data not shown) for glutamate concentrations between 10^{-11} M and 10^{-5} M, both in the presence and absence of added calcium.

As the present results represent the first evidence of the activation of guanylate cyclase by any neurotransmitter in a

broken cell preparation, the production of cyclic GMP from GTP following glutamate treatment was verified using an alternative assay procedure in which [α -³²P]GTP was used as the substrate. Essentially similar results were obtained (Fig. 4).

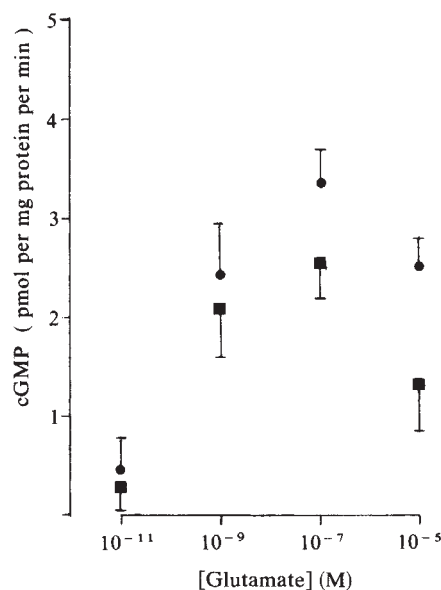


Fig. 2 Increase in guanylate cyclase activity after L-glutamate stimulation in the presence and absence of calcium. Membrane preparations were isolated, incubated and cyclic GMP extracted as described in Fig. 1 legend. ●, EGTA (1 mM); ■, Ca²⁺ (1 mM).

HPLC followed by TLC of control and phosphodiesterase-treated cyclic GMP column eluate fractions, confirmed that the radioactive product was 3', 5'-cyclic GMP. Glutamate stimulation of adenylate and guanylate cyclase activities has also been observed for an isolated membrane preparation prepared from the body-wall muscles of a second species of dipteran insect, *Lucilia sericata* (data not shown).

We have made a preliminary investigation of the pharmacological specificity of adenylate and guanylate cyclase. The neutral amino acid alanine was found to be totally inactive in stimulating either adenylate or guanylate cyclase activities. However, inclusion of alanine in the incubation medium reduced considerably the level of nonspecific interference in the cyclic GMP radioimmunoassay, therefore alanine was included at a low concentration in the assay buffer. α -Ketoglutaric acid, a precursor of L-glutamate, was also found to have no stimulatory effect. The structurally related amino acids aspartate and cysteine sulphinate were also tested and found to possess agonist activity but of a lower potency than glutamate. We also determined the cation requirements necessary for measurable cyclase activity. Although Ca^{2+} is not required in the final incubation medium, it must be included in the isolation buffer. Preliminary studies suggest that either Mg^{2+} or Mn^{2+} may serve as the major cation in the cyclase assay buffer.

Although various authors^{7,8} have reported stimulation of guanylate cyclase activity by calcium in broken cell preparations, and Kakiuchi and co-workers⁹ have shown that in *Tetrahymena pyriformis* calcium activation of membrane-bound guanylate cyclase is calmodulin dependent, the present report of glutamate activation of guanylate cyclase in a broken cell preparation is in sharp contrast to the failure in both our own and various other laboratories to demonstrate activation by neurotransmitters of guanylate cyclase in cell-free preparations. Our present results agree with previous observations in that guanylate cyclase can be activated by calcium, but differ from previous reports in that we have demonstrated an additional glutamate-sensitive component which may be activated by this neurotransmitter in the absence of calcium. It is not clear whether the neurotransmitter-sensitive guanylate cyclase reported here is unique to insect muscle or whether it represents a more general

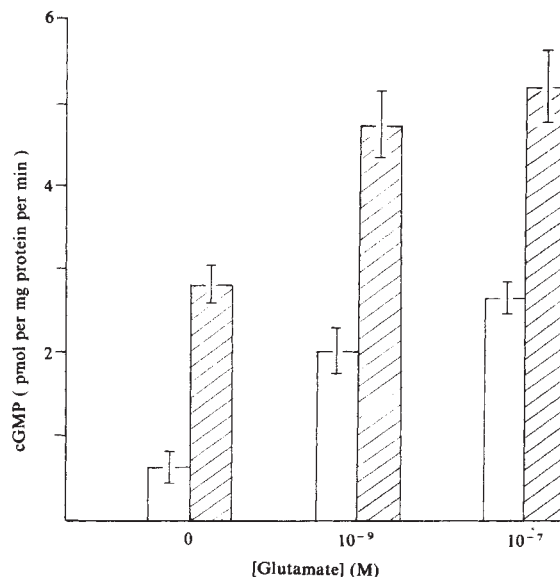


Fig. 4 Production of [α -³²P] cyclic GMP from [α -³²P]GTP. Membrane preparations were isolated and incubated as described in Fig. 1 legend except that [α -³²P]GTP (0.05 mol per mCi) was used as the substrate for the guanylate cyclase and the cyclic GMP produced during incubation was accordingly labelled with ³²P. Incubations were terminated as described in Fig. 1 legend and after evaporation to dryness, samples were extracted with 1 ml extraction buffer containing 1 nmol cyclic GMP to act as a marker during HPLC. A 0.5-ml sample was introduced to the chromatography column (Whatman SAX; 1.0 × 30 cm) and the chromatograph developed by isocratic elution using 0.005 M NaH_2PO_4 buffer, pH 4.2. The column eluate was continuously monitored for UV absorbance at 254 nm and 500- μ l samples collected using a Gilson fraction collector and monitored for radioactivity. The 'cyclic GMP' peak as determined by the UV absorbance of the added standard was found to correspond to a peak of radioactivity and the yield of [α -³²P] cyclic GMP calculated by determining radioactivity recovered from the column after injection of radioactive standards. Representative samples of 'cGMP' column fractions were pooled and evaporated to dryness under N_2 at 55 °C. Samples were then taken up in a small volume (20 μ l) of distilled water; 5 μ l was applied directly to TLC plates (silica gel GF, 250 μ m, with fluorescent marker) and the remainder incubated for 30 min with a high concentration of 3'5'-cyclic nucleotide phosphodiesterase (Sigma). This incubation was stopped by adding 5 vol of acidified ethanol. Samples were again evaporated to dryness under nitrogen at 55 °C and then taken up in 15 μ l distilled water; a 5- μ l sample was applied to a TLC plate together with the untreated sample and suitable cyclic GMP and GMP standards. The chromatogram was developed in *n*-butanol/ethyl/acetate/methanol ammonia/water (5:3:6:4:2). The developed chromatogram was monitored for UV absorbing spots and then 1-cm strips were scraped off and the radioactivity counted in a liquid scintillation counter. This procedure confirmed that the radioactive product co-chromatographed with cyclic GMP after phosphodiesterase treatment co-chromatographed with GMP. Open columns, with added EGTA; cross-hatched columns, with added Ca^{2+} .

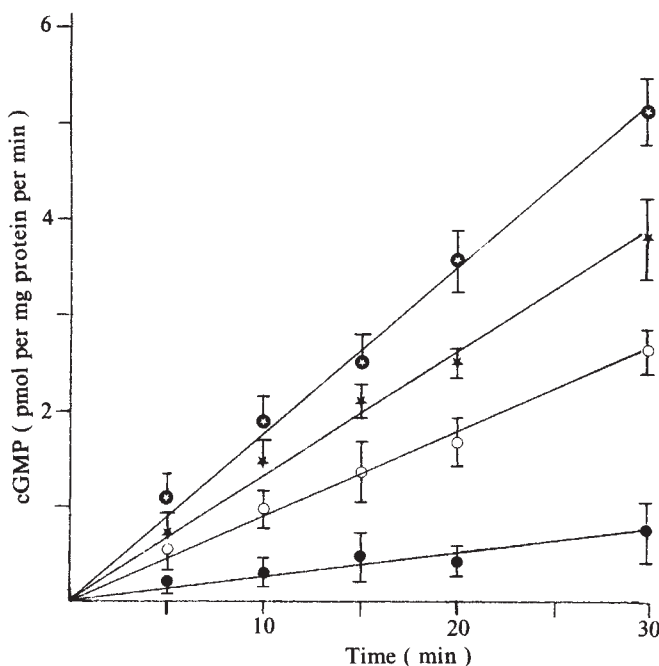


Fig. 3 Time of production of cyclic GMP by an isolated muscle membrane preparation in the presence and absence of added glutamate. Membrane preparations were isolated, incubated and cyclic GMP extracted as described in Fig. 1 legend, except for the variation in incubation time. ●, EGTA (1 mM); ○, EGTA (1 mM)+glutamate (10^{-7} M); ★, Ca^{2+} (1 mM); ☆, Ca^{2+} (1 mM)+glutamate (10^{-7} M).

system present in other tissues for which appropriate assay conditions have not yet been found.

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Single Ca^{2+} -activated nonselective cation channels in neuroblastoma

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Recent work suggests an important role for intracellular agents in controlling ion channels in the membranes of nerve cells and other excitable tissues. Calcium ions^{1,2}, cyclic nucleotides³ and protein kinases^{4,5} can all act on the inner surface of the membrane to influence ion channel activity. Earlier studies of these effects on intact cells could not, however, effectively control or measure conditions on the inner membrane surface. The technique of recording from detached membrane patches^{6,7} permits free access to the intracellular face of ion channels and makes it possible to study them in isolation from the many components of the cytoplasm. With this technique, I have studied the response of membrane patches from neuroblastoma cells to intracellular Ca^{2+} ions, and have found a class of nonselective cation channels activated by micromolar concentrations of Ca^{2+} on the intracellular face of the membrane. These channels are almost equally permeable to Na^+ , K^+ , Li^+ and Cs^+ ions, but are practically impermeable to Ca^{2+} ions. Similar channels were first found recently in cultured heart cells⁸, where they probably account for the previously reported 'transient inward' current⁹. Their discovery in neuronal cells as well as heart cells suggests that this hitherto scarcely recognized channel species may be more widely distributed than previously supposed.

Voltage-clamp recordings were from inside-out^{6,7} and outside-out⁷ membrane patches of mouse neuroblastoma cells (N1E-115). Inside-out patches have their intracellular face exposed to the bath, while outside-out patches have their extracellular face exposed to the bath. The exposed surface can be readily superfused. Cells were subcultured onto glass coverslips 2–7 days before use; some cells were treated with prostaglandin E_1 (PGE_1) ($10\text{ }\mu\text{M}$; P-L Biochemicals) 2 days after subculture (L.-Y.M. Huang, personal communication). Voltage-activated Na^+ channels were observed in most patches. Normal behaviour of Na^+ channels served to indicate that patches were not sealed over; rounded channel currents usually indicate that the membrane has sealed over to form a closed vesicle⁷. Other channels observed were Ca^{2+} -activated K^+ -selective channels and various voltage-sensitive outward current channels. The Ca^{2+} -activated nonselective channels were observed in about 1 out of 20 patches from cells not treated with PGE_1 , and about 1 out of 5 patches from treated cells.

Channel opening and closing events like those in Fig. 1 were seen in inside-out or outside-out patches when the intracellular face was bathed in $1\text{--}50\text{ }\mu\text{M}$ Ca^{2+} . These currents had a reversal potential close to 0 mV in a variety of ionic conditions (see below), and a conductance of $22 \pm 1.5\text{ pS}$ (s.e.m., $n = 6$) at 24°C , with 125 mM NaCl outside and 125 mM KCl inside.

Channel opening events occur in clusters, and their kinetics indicate a complex scheme for channel gating. A cluster of channel openings and closings may last on the order of 30 s . Within such a cluster, there are opening events lasting on the order of $50\text{--}200\text{ ms}$. One of these opening events may be interrupted by brief closing events (see, for example, the -70 mV trace in Fig. 1). Clusters of openings are sometimes separated by prolonged closings of many minutes. This

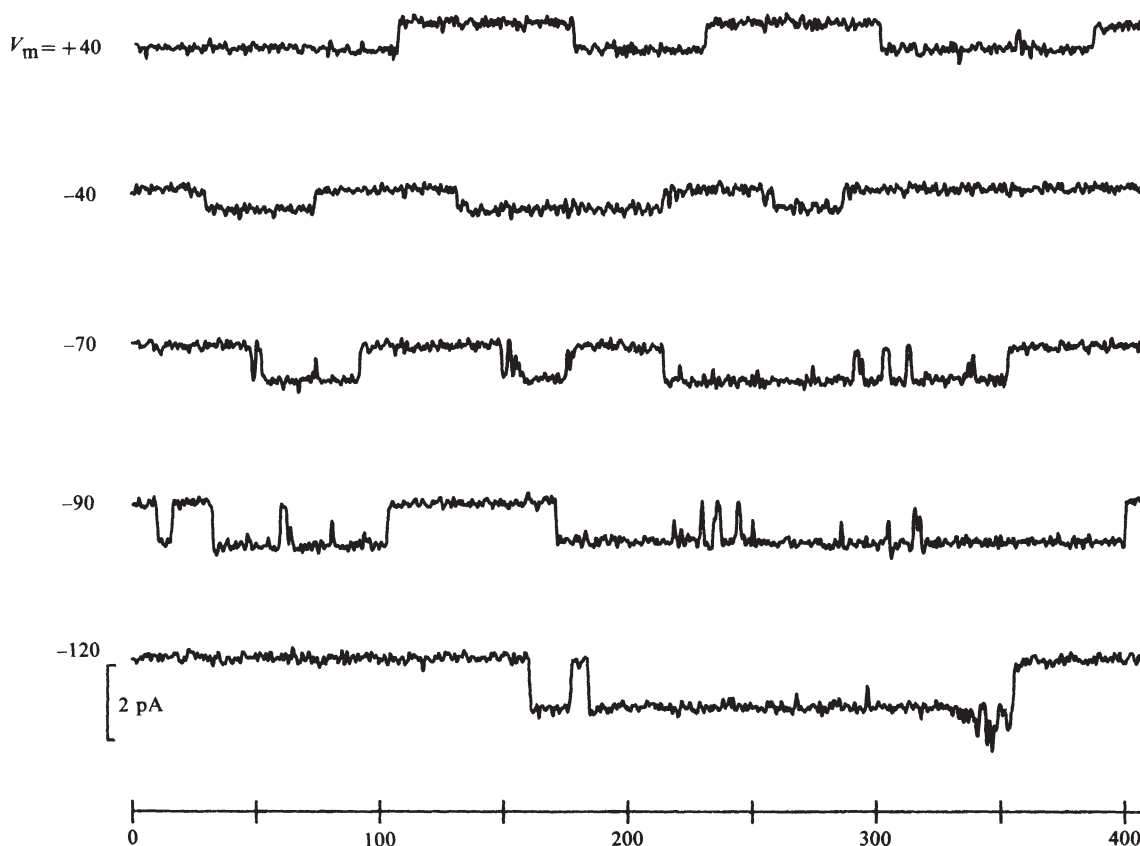


Fig. 1 Ca^{2+} -activated nonselective cation channel currents. Outward current is always defined as current from the intracellular to the extracellular side of the membrane, and is indicated as upwards. Membrane potentials are always specified as the potential on the intracellular side of the membrane, relative to the extracellular side. Channel currents are outward in the $+40\text{ mV}$ trace, and inward in all the traces at negative membrane potentials. Records are from an outside-out patch. Extracellular (bath; in mM): 125 LiCl , 2 CaCl_2 , 1 MgCl_2 , 10 HEPES , 70 dextrose , $\text{pH } 7.2$. Intracellular (pipette): 125 NaCl , 1 MgCl_2 , 10 HEPES , 70 dextrose , $5\text{ EGTA} + 4.925\text{ CaCl}_2$ (free $[\text{Ca}^{2+}] = 10\text{ }\mu\text{M}$), $\text{pH } 7.2$. All Ca^{2+} -EGTA buffers were computed with a K_{app} (at $\text{pH } 7.2$) $= 1.48 \times 10^{-7}\text{ M}$ (ref. 13, corrected for hydrogen ion activity). Temperature 22°C . Low-pass filtered at 500 Hz with an 8-pole Bessel characteristic.

behaviour indicates gating which is more complex than a single first-order transition between an open and a closed state. Gating of the channels is not strongly voltage dependent over a range from -120 to $+80$ mV.

Intracellular Ca^{2+} concentrations of $\geq 1 \mu\text{M}$ reversibly activate these channels (Fig. 2a), as can be seen by changing $[\text{Ca}^{2+}]$ on the exposed (intracellular) surface of an inside-out patch. If Ca^{2+} is removed (solution changed to 5 mM EGTA, 0 Ca^{2+} , 1 mM Mg^{2+}), the channel openings become less frequent and eventually cease (complete disappearance sometimes takes 1–2 min). When intracellular Ca^{2+} is restored, the channels begin to open almost immediately. This whole process can be repeated several times on a single patch, using the perfusion system shown in Fig. 2b. Solutions can be changed rapidly by moving the patch pipette among several continuously flowing perfusion streams.

The channel permeation selectivity was studied in outside-out patches, with 10 or 50 μM free $[\text{Ca}^{2+}]$ on the intracellular side (that is, in the pipette) to activate the channels. Two results argue against a significant anion permeability of the channels. First, replacing all but 3 mM of Cl^- by aspartate on one or both sides of the membrane changed neither the reversal potential nor the unit conductance. Aspartate is a much larger anion than chloride, and might be expected not to pass through the channels as easily. Second, channel currents in the presence of a salt gradient show approximately perfect selectivity for cations. In a fivefold salt gradient (25 mM NaCl extracellular, 125 mM NaCl intracellular; osmolarity preserved with dextrose), a perfectly cation-selective channel should reverse at -41 mV, and a perfectly anion-selective channel at $+41$ mV (22 °C). In such conditions, I found that these currents reverse within 2 mV of -41 mV.

Several alkali metal cations and Ca^{2+} were tested for permeation through the channel. All of the alkali metals permeated nearly as well as Na^+ . To obtain a complete current-voltage (I - V) relationship over a wide range of membrane potentials, a voltage ramp was applied to the membrane. Figure 3a shows a single record of membrane current in response to such a ramp (average leak current has been subtracted). When the channel opens, the current changes abruptly from the leakage level (zero in the leak-subtracted records) to the open channel level. For as long as the channel remains open, the current follows the I - V relationship for the open channel. A complete I - V relationship for the open channel may be obtained by extracting all the open channel segments from many records and averaging

them point by point for each voltage (points from one voltage are averaged only with other points from the same voltage, see Fig. 3b). The leakage current is obtained by a similar process.

The ramp clamp method has several advantages over measuring channel currents at many fixed potentials. Channel openings are small and difficult to see at fixed potentials near their reversal potential, but can readily be seen in ramp clamp records as an inflection in the slope of the current. Also, although it is sometimes difficult to distinguish between the opening of an inward current channel and the closing of an outward current channel at a fixed potential, the open channel is unambiguously identified in a ramp clamp by the increased slope of the I - V curve. Finally, a complete, continuous I - V curve can be obtained quickly, and without holding the membrane at depolarized potentials for prolonged periods (which often causes membrane breakdown). Open channel I - V curves derived from ramp clamp data agree well with those derived from measurements of fixed-potential records. Ascending and descending ramps give similar results.

The open channel I - V curve in symmetrical 125 mM Na^+ has a slope conductance at 0 mV of ~ 17 pS (22 °C), and shows a small outward rectification at positive potentials (Fig. 3b). K^+ goes through the channel just as easily as Na^+ : the reversal potential with Na^+ outside and K^+ inside is still 0 mV. Cs^+ and Li^+ show only slightly lower permeability than Na^+ and K^+ , with a reversal potential of -8 mV (Cs^+ or Li^+ outside, Na^+ inside) and a slope conductance at hyperpolarized voltages of ~ 12 pS. Ca^{2+} (82 mM, outside; Na^+ inside) has a very low permeability (if any); the channel current remains outward over a range of hyperpolarized voltages (Fig. 3b).

Neuroblastoma cells have at least one other type of Ca^{2+} -activated channel, a Ca^{2+} -activated K^+ channel (unpublished observations). This channel is similar in its properties to the Ca^{2+} -activated K^+ channels described by Marty¹⁰ in chromaffin cells and by Pallotta, Magleby and Barrett¹¹ in cultured rat muscle cells. Even though the Ca^{2+} -activated K^+ channels have about 10 times the conductance of the Ca^{2+} -activated nonselective channels, they are much more selective in their permeation.

Recently, it was reported that pressure-injection of CaCl_2 in snail neurones evokes an inward current¹². However, this Ca^{2+} -activated inward current is not affected by replacing all the extracellular NaCl with sucrose, and may thus have a different ionic basis from the Ca^{2+} -activated nonselective cation channel currents described here.

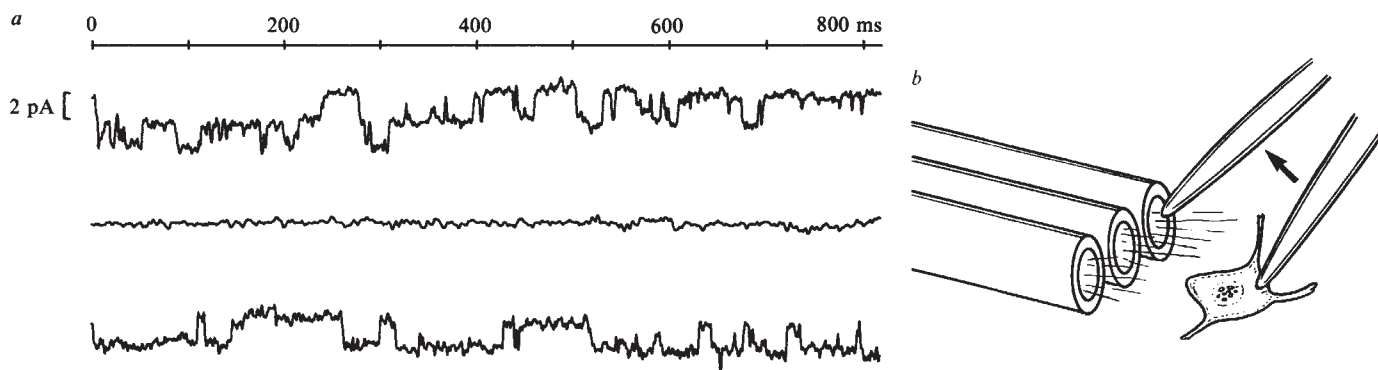


Fig. 2 a, Ca^{2+} -dependent activation of channel currents. A series of records was collected with different intracellular $[\text{Ca}^{2+}]$. Top trace: channel currents with 1 μM free $[\text{Ca}^{2+}]$ solution perfusing the intracellular side. Middle trace: current record after 2 min perfusion with 0 free $[\text{Ca}^{2+}]$ solution (that is, 5 mM EGTA, no added Ca^{2+}). Bottom trace: channel currents immediately after return to 1 μM free $[\text{Ca}^{2+}]$. Solution changes were performed using a perfusion system like that shown in b. Temperature 25 °C; 300 Hz filtering. Data are from an inside-out patch with Na^+ saline outside (divalent ions: 3 mM MgCl_2 , 0.1 μM free $[\text{Ca}^{2+}]$ (5 mM EGTA buffer)) and K^+ saline inside (divalent ions: 1 mM MgCl_2 , 1 μM free $[\text{Ca}^{2+}]$ or 0 free $[\text{Ca}^{2+}]$ (5 mM EGTA buffer)). Membrane potential is -60 mV. b, Diagram of perfusion apparatus for rapid solution changes. Solutions flow continuously out of perfusion pipettes (i.d. $\sim 70 \mu\text{m}$) into the bath at a rate of 5–50 linear $\mu\text{m s}^{-1}$ (estimated from the velocity of small particles in the centre of the flow stream). After detaching the membrane patch from the cell, the patch pipette is moved completely into the outflow of one of the perfusion pipettes. Solution changes can be readily performed by moving the patch pipette into a different perfusion stream. Moving the pipette takes about 1 s; the solution change at the membrane is quite rapid, as judged from the immediate response of Ca^{2+} -activated K^+ channels to perfusion of Ca^{2+} solutions. This apparatus was used for changing internal $[\text{Ca}^{2+}]$ in experiments with inside-out patches, and for changing external cations in selectivity experiments with outside-out patches.

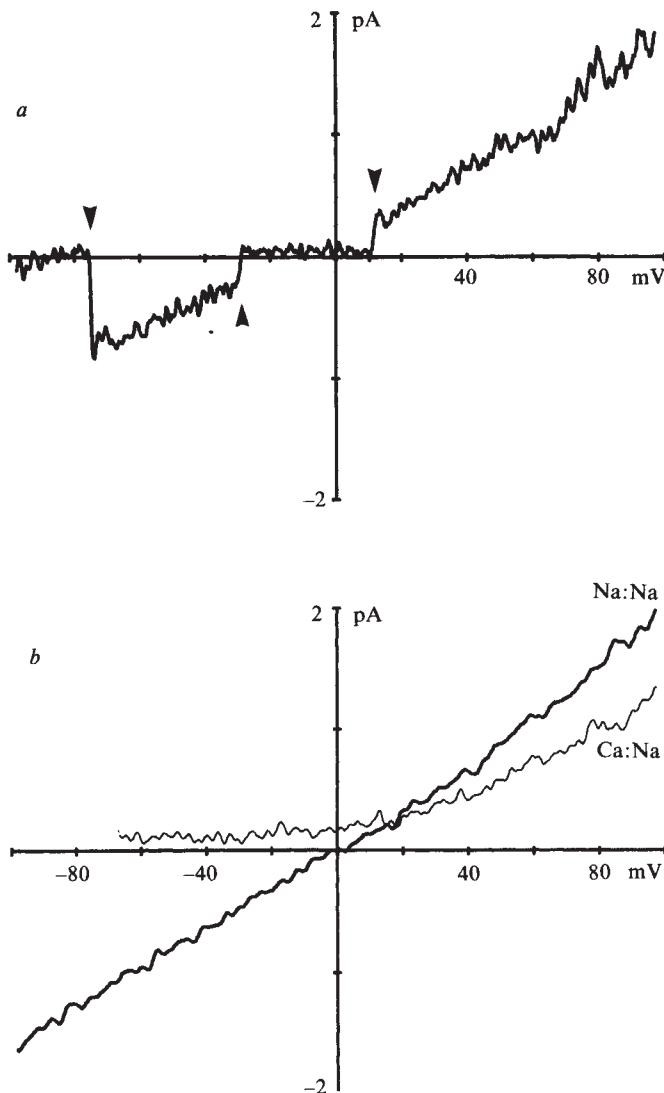


Fig. 3 Open channel I - V relationship obtained with ramp voltage clamp. *a*, Single current record from outside-out patch with Li^+ saline outside, Na^+ saline (with $10 \mu\text{M}$ free $[\text{Ca}^{2+}]$) inside. The average leak current from 32 records has been subtracted (averaging method is described in the text). The arrows indicate channel transitions: the first arrow indicates a channel opening, the second a closing, and the third an opening. The residual current when the channel is closed is due to imperfect leak subtraction. A ramp from -100 to $+100$ mV was performed in 410 ms. Temperature 22°C ; 300 Hz filtering. *b*, Average open channel I - V curves. Open channel segments of records like that in *a* were identified to the computer, which averaged these segments point by point for each voltage. An average leakage I - V was subtracted from the open channel I - V (the leakage I - V was also collected by selective averaging, and, in this experiment, the leakage current was smaller than a single open channel current). The $\text{Ca}:\text{Na}$ I - V was obtained with (in mM): 82 CaCl_2 , 10 HEPES, 70 dextrose, pH 7.2 extracellular, and Na^+ saline ($10 \mu\text{M}$ free $[\text{Ca}^{2+}]$) intracellular. Temperature 22°C . In measurements of the $\text{Ca}:\text{Na}$ I - V , channel opening events were observed at potentials as low as -10 mV (size was $+0.2$ pA); below this potential, the currents were obtained from the average of several records which appeared to have a channel open during the whole trace.

Channels very similar to those described here have recently been found in primary-cultured rat heart cells⁸. They are activated by intracellular Ca^{2+} , not much affected by voltage, have a size of ~ 23 pS at 22°C and pass Na^+ and K^+ equally well. They may be responsible for the 'transient inward' current which produces oscillatory depolarizations (causing arrhythmias) in heart cells exposed to high Ca^{2+} or to Na^+-K^+ -pump inhibitors⁹. The finding that these channels, appreciated only

for their role in the physiological dysfunction of cardiac cells, also appear in neuronal cells suggests that this channel species may be more widespread in both its occurrence and significance.

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Human tumour antigens defined by cytotoxicity and proliferative responses of cultured lymphoid cells

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The long-term goal of many laboratories has been to develop cellular reagents having specific reactivity against human tumour cells. Such immune cells should prove useful for defining the antigenicity of human malignancies and may have important therapeutic potential, as has been clearly shown in some animal models¹. Here we describe methods of initiating continued lymphocyte cultures (CLC) having specific anti-tumour reactivity using conditioned media containing interleukin-2 (IL-2).

Direct cytotoxicity assays show that $\sim 30\%$ of patients have specific reactivity against autologous tumours²⁻⁴. It was anticipated that such *in vivo* sensitized cells would express receptors for IL-2 and thereby be selectively susceptible to growth *in vitro* in the presence of IL-2 (ref. 5). Blood lymphocytes were prepared from heparinized blood samples taken from cancer patients on the morning of surgery, before the administration of any narcotic agent. Mononuclear cells were isolated by flotation on Ficoll-Hypaque (Lymphocyte Separation Medium, Litton Bionetics, Maryland). Adherent cells were removed by culture in flasks (75 cm^2) at 37°C overnight. The lymphocytes ($3 \times 10^5 \text{ ml}^{-1}$ in RPMI+20% heat-inactivated pooled normal human serum) were grown (without *in vitro* stimulation with tumour cells) in optimal quantities of different conditioned media containing IL-2 reactivity. The resulting cultured cells were tested for cytotoxicity against a range of target cells, including freshly isolated autologous and allogeneic tumour cells. CLC were also tested against the K562 cell line to determine the possible presence of natural killer (NK) cells. The cultured cells showed cytotoxicity against both autologous and allogeneic tumour targets and, in at least some conditioned media, high levels of cytotoxicity were also found against K562 (Table 1). There was no evidence of selective reactivity against the autologous tumour alone and frequently, cytotoxicity against allogeneic tumours of unrelated types exceeded that against autologous tumours. Similar data have been obtained by other workers⁶⁻⁹. The results are compatible with the presence within such cultures of several different types of effector, including polyclonally activated T¹⁰⁻¹² and natural killer (NK) cells^{10,13}.

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Table 1 Cytotoxicity of lymphocytes from cancer patients after culture for 21 days in conditioned media containing IL-2

Patient (site of tumour)	Type of medium	% Cytotoxicity against:		
		Autologous tumour	Allogeneic tumour	K562
Tu 3 (ovary)	H-CM	3.3	10.9*	51.6*
Tu 4 (parotid)	H-CM	28.9*	35.5*	87.6*
Tu 5 (parotid)	H-CM	8.6*	4.0	75.0*
Healthy donor	S-CM	15.9*	18.7*	31.3*
Tu 85 (lung)	S-CM	15.9*	18.7*	31.3*
Tu 58 (colon)	S-CM	13.6*	0	0
Tu 55 (colon)	S-CM	8.5*	33.7*	23.8*
Tu 131 (parotid)	LF-CM	18.4*	18.2*	0
Tu 57 (colon)	LF-CM	18.4*	5.2	8.1*
Tu 139 (gastric)	LF-CM	10.6*	20.8*	2.8

H-CM, from thoracic duct lymphocytes (phytohaemagglutinin (PHA) content $0.04 \mu\text{g ml}^{-1}$ by haemagglutination); S-CM, from spleen lymphocytes (PHA content $2 \mu\text{g ml}^{-1}$); LF-CM, from tonsillar lymphocytes depleted of PHA by passage through an immunoabsorbent column of rabbit anti-PHA (no PHA detectable). Tumour tissue was received within 3 h of removal from patients. Tumour cells, tumour-infiltrating lymphocytes and macrophages were isolated from enzymatically dispersed cell suspensions by methods described in detail elsewhere²¹. All populations showed high viability ($>95\%$ by Trypan blue exclusion) and contamination with 'inappropriate' cells was $<10\%$ as judged by morphological examination and histochemical staining^{1,20}. Preparations were either used immediately as stimulators in MLTC, or frozen in RPMI 1640 supplemented with 20% human serum and 10% dimethyl sulfoxide (2.5×10^6 cells in 2 ml) by constant-rate freezing at 1°C min^{-1} to -30°C and stored over liquid nitrogen. Allogeneic tumours were, as far as possible, matched for cytotoxicity testing with the autologous tumours with regard to tumour site and histology. Cytotoxicity at effector/target ratio of 5:1 was determined after overnight incubation in fresh medium¹³, for each case.

* $P < 0.05$, Mann-Whitney U-test.

In an attempt to induce more specific reactivity, blood lymphocytes were sensitized *in vitro* in mixed lymphocyte-tumour cultures (MLTC). Responder cells (1×10^7 in 10 ml RPMI plus 20% human serum) were mixed with irradiated ($3,000 \text{ R}$) tumour stimulators (2×10^6 in 2 ml medium) and cultured at 37°C for 6 days in 25 cm^2 culture flasks kept upright. MLTC was considered positive when mean ^3H -thymidine incorporation of stimulated cultures exceeded by at least threefold that of controls and clear dose-dependence of stimulation was apparent, and when blast cells were recognizable in bulk cultures. The factors critical for induction of high frequency of stimulation by autologous tumour cells have been considered elsewhere¹⁴. In the present experiments, positive MLTC was defined in 15 of 22 cases. Surviving lymphocytes were expanded by adding IL-2-containing media as described above, and these (MLTC-CLC) were tested for cytotoxicity after 10 and 21 days in culture. Typical results for such cultures are shown in Fig. 1. While some preferential reactivity towards autologous

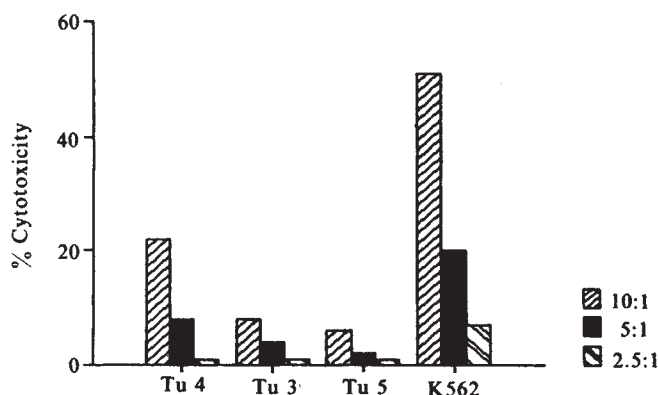


Fig. 1 Cytotoxicity of MLTC-positive cells grown in H-CM containing IL-2 for 17 days. Effectors were cultured in the absence of IL-2 for 24 h before testing and tested in a single assay at effector/target ratios of 10:1, 5:1 and 2.5:1 against the autologous tumour Tu 4 (parotid), and allogeneic tumours Tu 3 and 5 (ovarian ascites). The K562 cell line was used in measures of NK activity; cytotoxicity against the latter represented the major cytolytic potential in most of these cultures. Spontaneous ^{51}Cr -release was: Tu 4, 21%; Tu 3, 11%; Tu 5, 14%; and K562, 7%.

tumours was induced in MLTC-positive cultures, CLC still had significant reactivity against allogeneic tumours and high cytotoxicity against K562. Thus, they were not appreciably more selective in their reactivity than unstimulated CLC.

To select for specific anti-tumour reactivity, blasts were isolated from MLTC on day 6 of culture on discontinuous gradients of Percoll in RPMI plus 20% human serum. Gradients were prepared in 15-ml tubes (No. 2095, Falcon Plastics, Maryland) and comprised 40% Percoll (5 ml); 35% (1.5 ml); 31.5% (2.5 ml) and 26% (2.5 ml). Samples were applied to the gradient in 1 ml medium and centrifuged at $550g$ for 30 min at room temperature. Blast cells were recovered from the 31.5% interface, lower numbers (contaminated with small lymphocytes) also being found at the 35% interface. Cells were washed twice in RPMI 1640 and cultures initiated at a concentration of 3×10^5 cells ml^{-1} in RPMI plus 20% human serum supplemented with 10% conditioned medium containing IL-2 activity. These MLTC-blast-CLC were tested for cytotoxicity (4 h ^{51}Cr -release assay) and proliferation after 8–29 days of culture in conditioned medium. Of 15 MLTC-blast-CLC, 13 showed selective lysis of autologous tumours (Table 2). Reactivity was apparent at low effector/target ratio and was dose dependent. By contrast, significant reactivity was only rarely recorded against at least two allogeneic tumour targets (Table 2). In many cases, cross-reactivity tests showed that the control targets were susceptible to lysis by appropriate autologous MLTC-blast-CLC. Following blast isolation, many cultures

Table 2 Cytotoxic reactivity of MLTC-blast-CLC grown in H-CM

Tumour no.	Diagnosis	% Cytotoxicity (effector/target ratio, 5:1) against:		
		Autologous tumour	Allogeneic tumour (no.)	K562
7	Lung cancer	28.4*	2.0 (11)	10.1*
8	Cancer of gingiva	100*	5.7 (7)	19*
11	Lung cancer	23.7*	4.5 (7)	0
12	Melanoma	18.4*	1.4 (11)	7
15	Lung cancer	38.5*	1.4 (17)	1
17	Lung cancer	42.4*	0.4 (15)	2.7
25	Breast cancer	34.6*	8.7 (26)	21.8*
26	Breast cancer	15.5*	2.2 (25)	16.3*

Spontaneous release of ^{51}Cr from fresh tumour cells was $<25\%$ in a 4 h assay; K562 showed spontaneous release between 11% and 21% of total. Tests were performed on the same day against autologous tumour, blood monocytes and PHA-induced lymphoblasts and against at least two allogeneic tumour preparations matched, as far as possible, for tumour site and histology. No significant cytotoxicity was found against six autologous lymphoblasts (cytotoxicity ~ 5.1 to 4.7%), but was evident against 1/7 blood monocytes (tumour 12; 11.4%) and in 4/41 cross-reactivity tests with 15 MLTC-blast-CLC against allogeneic tumour ($\sim 4.3\%$ to 18.6% cytotoxicity).

* $P < 0.05$.

showed only low levels of cytotoxicity against the K562 cell line. The ability to reduce NK-like activity by density separation facilitated the interpretation of MLTC-blast-CLC killer function against autologous tumour. However, as an additional control, CLC were initiated from blasts induced by co-cultivation of tumour cells with allogeneic lymphocytes in seven cases. Five allogeneic MLTC-blast-CLC showed significant proliferation and cytotoxicity that was specific for the inducing tumours and levels of lysis were similar to those of autologous MLTC-blast-CLC against the same targets. Some cultures (7, 8, 25 and 26 of Table 2) continued to show significant NK activity against K562. The reason for the variability of NK levels among different CLC is not clear but cannot be related to levels of proliferation of particular MLTC.

Proliferative responses of MLTC-blast-CLC were assessed in primed lymphocyte tests. Cultures were restimulated with autologous and allogeneic cells in 48-h tests; 15 MLTC-blast-CLC showed a significant increase in ^3H -thymidine uptake on restimulation with autologous tumour cells. The results of a series of assays are given in Table 3. MLTC-blast-CLC from a patient with squamous cell carcinoma (SCC) of the lung showed significant proliferation after restimulation with

Table 3 Primed lymphocyte test of MLTC-blast-CLC 17 (squamous cell carcinoma of lung)

Stimulator	No. of responders per well			
	1×10^4	5×10^3	2.5×10^3	1.25×10^3
CLC alone	1,115	530	620	360
Tumour 17	7,302*	9,314*	5,781*	4,069*
SCC 16	3,447*	2,100*	2,687*	561
Tumour macrophages 17	4,443*	1,553*	698	187
Monocytes 17	892	649	549	304
Lymphocytes 16	1,022	1,241	1,145	736
Lymphocytes 15	1,625	1,319	1,448	480

Restimulation of MLTC-blast-CLC 17 with autologous (tumour, monocytes and macrophages 17) and allogeneic cells. Decreasing numbers of responder cells were cultured for 48 h with a constant number (5×10^4 per well) of stimulator cells. Uptake of ^3H -thymidine was measured after a 6-h pulse with 0.5 μCi radiolabel per well. Uptake of ^3H -thymidine by stimulators was <100 counts per well and this value was subtracted from stimulated cultures. Stimulators tested were autologous tumours (squamous cell carcinoma of lung), autologous tumour-derived macrophages, autologous blood monocytes, allogeneic squamous cell carcinoma of lung, and lymphocytes from allogeneic tumour donors 16 (ref. 15). Further testing of the MLTC-blast-CLC 17 line showed that it was stimulated neither by four allogeneic lymphocytes nor by eight allogeneic non-SCC tumours.

* $P < 0.05$, Student's *t*-test.

autologous tumour. Less proliferation was apparent with tumour macrophages as stimulators and no increased ^3H -thymidine incorporation was induced by autologous blood monocytes, lymphocytes or a panel of allogeneic lymphocytes and monocytes. We expected that primed lymphocyte test responses might be restricted by histocompatibility at the Ia region. This restriction has affected proliferative responses to soluble antigens in mouse^{15,16} and man¹⁷. In our study, proliferative responses to malignant cells in MLTC-blast-CLC appeared to be limited by tumour site and histology, not by HLA. Three squamous cell carcinomas of the lung (one of which is given in Table 2) showed complete cross-reactivity in that allogeneic SCC also induced significant restimulation while no stimulation was induced by small cell carcinoma or adenocarcinoma of the lung, tumours of the ovary or colon, or blasts from acute myelogenous leukaemia. Similar results were obtained for CLC from nine patients with adenocarcinoma of the breast. Allogeneic tumours induced variable degrees of restimulation but levels of ^3H -thymidine incorporation were often comparable with those induced by autologous tumour. The panel of stimulator cells is now being expanded to investigate further the specificity of this primed lymphocyte test response, but the data contrast with the high individual specificity seen in cytotoxicity assays. These results may be explained by HLA restriction of killing to HLA-A, -B or -C (ref. 18) or by individual tumour-associated antigens, whether or not they involve HLA restriction. The question remains as to whether the lymphocyte-stimulating and cytotoxic T-cell target antigens are different¹⁸.

We have thus obtained cultured cells having specific anti-tumour reactivity by adopting approaches which, in our laboratory and in others, have been effective in inducing cultured cells having specificity for alloantigens¹⁸⁻²⁰. Critical features of these experiments were: (1) the use of MLTC-positive cultures (while cultures could be initiated from MLTC-negative individuals, no specific reactivity was found in these CLC); (2) isolation of blast cells—without this, the cultured cells showed features of polyclonal T-cell activation and high NK activity.

Using these MLTC-blast-CLC, we have demonstrated selective lysis of autologous tumours and restimulation in primed lymphocyte test assays. The nature of the antigens against which these responses were directed is now being investigated. Although there is no evidence that reactivity of specific CLC was directed against HLA-D/DR (failure to lyse autologous monocytes or undergo restimulation by monocytes and lymphocytes), the induction of autoreactivity to other 'normal' antigens, such as organ-specific or differentiation markers, cannot be excluded. We envisage that access to further normal

control tissues should aid the investigation of these reactions, as should the availability of T-lymphocyte clones.

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Amplification and rearrangement of *onc* genes in mammalian species

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Related eukaryotic species usually contain the same number of copies per cell of a given 'unique sequence' gene. In the few described exceptions, such as the preproinsulin^{1,2} and globin genes³⁻⁷, one or two additional copies per cell have been found in several related species, suggesting that germ-line amplification occurred millions of years ago in a common progenitor of these species. The transforming (*onc*) genes of retroviruses are a group of evolutionarily conserved genes for which at least 10 distinct members have been described (for a review see ref. 8). Sequences related to each *onc* have been identified in all vertebrate species tested, usually as one copy per haploid genome, although the rat has at least two different genes homologous to the *onc* of the rat-derived Harvey murine sarcoma virus (Ha-MuSV)*. In screening genomic DNA from several rodent species for sequences related to the *onc* of Ha-MuSV and to the closely related (but distinguishable) *onc* of Kirsten (Ki) MuSV¹⁰, we have now found evidence for relatively recent amplification of these genes. We report here that *Mus pahari* apparently contains at least 10 copies of Ha-MuSV-type *onc*, whereas most other *Mus* species contain only one or two copies. Similarly, Chinese hamsters (*Cricetulus griseus*) have about six copies of the Ki-MuSV-type *onc* compared with only one copy in Syrian hamsters (*Mesocricetus auratus*).

In agreement with recently proposed nomenclature¹¹, the *onc* of Ha-MuSV has been designated v-Ha-ras, while the two homologous rat cellular genes which have been molecularly cloned are called c-Ha-ras1 and c-Ha-ras2 respectively. (In ref. 9, c-Ha-ras1 is called *sarclI* and c-Ha-ras2, *sarclI*. Sarc II

is called c-Ha-ras1 because it is the most highly conserved form of c-Ha-ras¹².) Each rat cellular gene is homologous to v-Ha-ras in ~0.9 kilobase (kb)⁹. In c-Ha-ras2 these homologous sequences are co-linear with v-Ha-ras, while c-Ha-ras1 has intervening sequences (introns; see Fig. 1). The rat c-Ha-ras1 gene, v-Ha-ras and v-Ki-ras (the *onc* of Ki-MuSV), each encode 21,000-molecular weight proteins (p21) which are biologically, immunologically and functionally related^{9,10,13-15}, although v-Ha-ras and v-Ki-ras DNA probes hybridize in stringent conditions to different restriction enzyme fragments of vertebrate genomic DNAs¹⁰. High levels of virus- or cell-encoded p21s can induce cellular transformation^{9,10,13}.

To detect and analyse the *ras* sequences in rodent DNA, *Eco*RI-digested embryo or tissue culture genomic DNAs from several inbred strains of *M. musculus*, as well as from other *Mus* species, rat and mink (a non-rodent), were electrophoresed, transferred to nitrocellulose paper and hybridized to a probe derived from either v-Ha-ras or v-Ki-ras (Fig. 1A, C). The v-Ha-ras probe detected the same *c-ras* fragments in all *M. musculus* and in *Mus castaneus* and *Mus poschiavinus* (Fig. 1A), while the pattern of *c-ras* fragments was distinctive for the DNAs from mink, rat, and the other *Mus* species. The most striking observation, however, was that, in contrast to the relatively few *c-ras* fragments in the other species, the DNA of a *M. pahari* cell line (Fig. 1, lane *m*) contained many fragments which hybridized strongly to the v-Ha-ras probe. We estimate that these fragments represent at least 10 copies per haploid genome. As the v-Ki-ras probe hybridized to only two fragments in *M. pahari* DNA (Fig. 1C), we conclude that the amplification in *M. pahari* consisted of c-Ha-ras sequences. (The multiple fragments seen in rat DNA using the v-Ki-ras probe are due, as noted previously¹⁰, to the presence of some non-*ras* viral sequences in the probe; hybridization of cloned human c-Ki-ras DNA¹² to rat DNA revealed only four *Eco*RI fragments; data not shown.) The amplification of the Ha-ras sequences in *M. pahari* was not a result of *in vitro* propagation of the cell line, as hybridization of genomic DNA from both the cell line and *M. pahari* embryos obtained from a different colony gave almost identical results (Fig. 1E). This result also suggests that the amplification is relatively stable in *M. pahari*. The transcriptional activity of these multiple c-Ha-ras sequences has not yet been determined, but the *M. pahari* cell line, which is not morphologically transformed, contains low levels of p21 comparable with those found in other normal tissue culture cells from many other vertebrate species¹⁶ (data not shown).

As the rat c-Ha-ras1 contains a 1.5 kb *Pvu*II fragment which serves as a useful marker for the gene (see diagram in Fig. 1), *Pvu*II-digested genomic DNAs were hybridized with the v-Ha-ras probe and with a probe derived from an intron of rat c-Ha-ras1 located within the 1.5 kb *Pvu*II fragment (Fig. 1B, D). For all *Mus* species the intron probe hybridized selectively to a 1.5 kb *Pvu*II fragment which co-migrated with the rat c-Ha-ras1 1.5 kb *Pvu*II fragment, whereas mink DNA did not contain this fragment (Fig. 1D). The intensity of the 1.5 kb fragment was similar in *M. pahari* and other cell DNAs. Hybridization of the v-Ha-ras probe to the genomic DNAs (Fig. 1B) revealed that *M. pahari* contained many more *Pvu*II fragments than did the other DNAs. These results strongly suggest that the gene carrying the intervening sequences is highly conserved among the rodents and that most or all of the additional copies of c-Ha-ras in *M. pahari* do not contain sequences homologous to the intron probe. The data from the non-rodent (mink) DNA also suggest that the intron sequences are less highly conserved than are the exon sequences of c-Ha-ras1.

The amplification of p21-related sequences is not limited to Ha-ras. Genomic DNA from Chinese hamster ovary (CHO) cells digested with *Eco*RI contained at least eight fragments which hybridized to the v-Ki-ras probe, whereas Syrian hamster DNA contained only one such fragment (Fig. 1F). Similar results have been obtained with *Bam*HI and *Kpn*I digestions

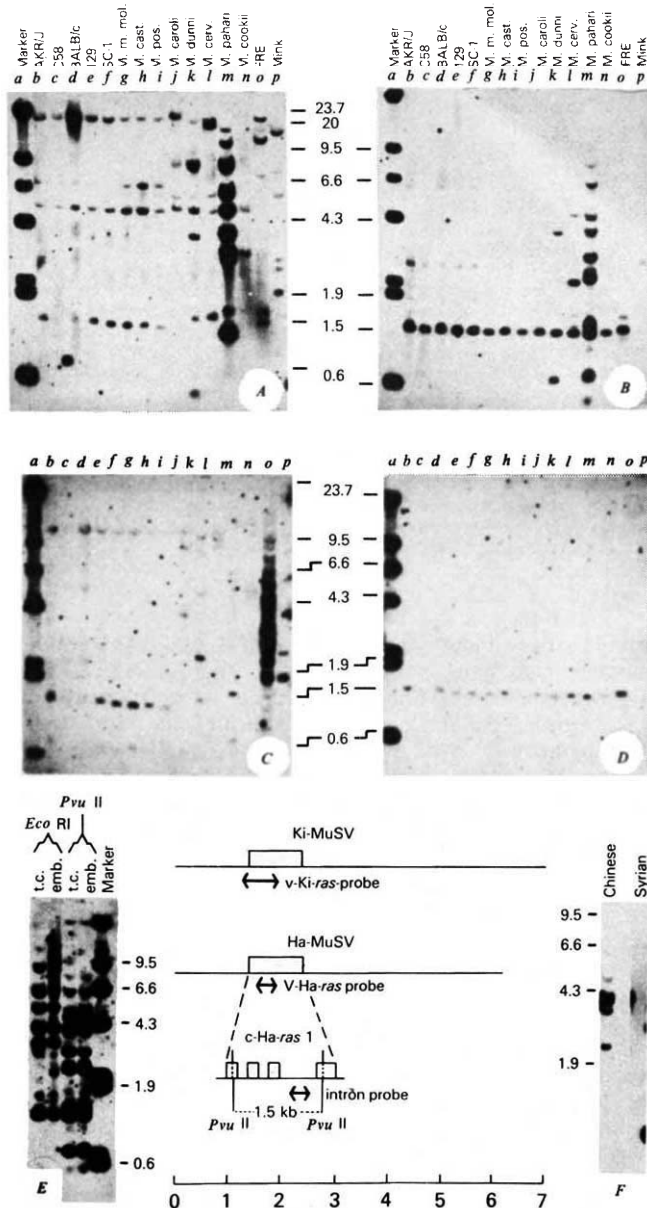


Fig. 1 Blot hybridization of genomic DNAs to *ras* probes. Restriction endonuclease-digested high molecular weight DNAs were electrophoresed in 0.5% agarose slab gels, transferred to nitrocellulose filters²⁶ and hybridized to cloned *ras* DNA labelled with ³²P by nick-translation²⁷. A and B, ³²P-labelled v-Ha-ras hybridized to *Eco*RI-digested and *Pvu*II-digested cell DNA respectively. C, ³²P-v-Ki-ras vs *Eco*RI-digested cell DNA; D, ³²P-intron of c-Ha-ras vs *Pvu*II-digested cell DNA. E, ³²P-v-Ha-ras vs *M. pahari* tissue culture (t.c.) and embryo (emb.) DNA. F, ³²P-v-Ki-ras vs *Eco*RI-digested hamster cell DNA. In A-D, AKR/J, C58, BALB/c and 129 DNAs were isolated from embryos, and other DNAs were isolated from tissue culture cells. M. m. mol, *Mus musculus molossinus*; M. cast., *M. castaneus*; M. pos., *M. poschiavinus*; M. cerv., *M. cervicolor*; FRE, Fischer rat embryo cells. Marker was ³²P-labelled *Hind*III-digested λ DNA. Fragment sizes are in kb. The *M. pahari* adult tail tissue culture line²⁸ (t.c. in E) was established in 1979 from a mouse provided by T. C. Hsu; the embryo DNA (emb.) was isolated from a mouse provided in 1981 by Richard Sage. In F, Chinese = CHO cells²⁹; Syrian = Syrian baby hamster kidney cells³⁰. In A, the probe has hybridized to four fragments of *M. musculus* DNA: 23, 7, 5 and 1.5 kb. In other experiments the v-Ha-ras probe consistently hybridized to the 23 kb fragment, but the hybridization to the other fragments was less consistent. The 7 and 5 kb fragments probably represent uncharacterized sequences homologous to c-Ha-ras. The 1.5 kb fragment probably represents c-Ki-ras sequences, as the v-Ki-ras probe consistently hybridizes to a 1.5 kb fragment (B). In the diagram, the relative locations of the *ras* sequences in the linear viral DNAs are indicated by a rectangle. The locations of sequences in the rat c-Ha-ras1 gene homologous to v-Ha-ras are shown by boxes; the spaces between these boxes represent introns. The arrow beneath each DNA indicates the location of sequences in each probe: the v-Ki-ras probe is the 0.6-kb *Sst*II/*Hinc*II fragment of clone HiHi-3 (ref. 10); the v-Ha-ras probe is clone BS-9 (ref. 9), and the intron probe is a 0.3 kb *Hinc*II fragment obtained by electroelution from a clone of c-Ha-ras1 in pBR322 (ref. 9).

(data not shown). As in the case of the *M. pahari* embryos, DNA from Chinese and Syrian hamster embryos resembled DNA from the cell lines of the respective species (data not shown).

Since closely related species contain only one or two copies of these amplified gene sequences, the *ras* copy number has probably increased relatively recently in *M. pahari* and in Chinese hamsters. The *ras* amplification therefore differs from other amplified unique sequence genes, such as globin and preproinsulin, in its high copy number and recent origin, from that of described orphans¹⁷ in its stability and in its affecting unique sequence genes, and from the transient nonchromosomal amplification of ribosomal genes during amphibian oogenesis¹⁸.

The structure of the amplified *ras* sequences remains to be established. The lack of homology to the intron of c-Ha-*ras*1 indicates that the amplified Ha-*ras* genes differ in structure from the c-Ha-*ras* gene with introns. The results suggest that these sequences may represent genes which lack introns entirely, as was the case in the rat c-Ha-*ras*2 gene, although the data reported here are compatible with other structures. If the amplified sequences lack introns, their structure would also resemble that of *c-mos*, the *onc* which gave rise to the transforming sequences of Moloney murine sarcoma virus¹⁹, and of an α -globin pseudogene which is present in mice and other species^{3,4,7}. The analogy of *c-mos* suggests that, unlike pseudogenes, the amplified *ras* sequences might encode a biologically active p21.

It is not known what conditions and molecular mechanisms led to the amplification of the *ras* genes, whether the amplified genes are clustered or dispersed, or what the biological consequences of this amplification may be. Analysis of the sensitivity of the *M. pahari* and Chinese hamster cells to the biological activity of p21, the distribution of the *ras* sequences within the cell genome, the structure of the amplified genes and their biological potential should, however, yield significant insights into these amplified sequences.

The amplification is probably not due to a general tendency of these animals to amplify genes as in *M. pahari* neither the closely related c-Ki-*ras* sequences nor the β -globin sequences were amplified (A. Leder, personal communication). The amplification might represent a genetic response to a non-infectious environmental agent, such as a chemical. Marked amplification of the dihydrofolate reductase (DHFR) gene has been induced in tissue culture cells in response to methotrexate; in cells with stable methotrexate resistance, the amplified DHFR genes are chromosomal and clustered²⁰. Alternatively, because transforming retroviruses arise by transduction of cellular *onc* genes⁸, the amplification might have arisen by this mechanism through putative endogenous retrovirus-like sequences or even through horizontal viral infection. In this model the *ras* sequences would be dispersed, as the termini of retroviral genomes resemble those of transposons and they integrate at multiple sites^{21,22}. In addition, endogenous retroviral genomes are usually found in multiple copies and may be absent from closely related species²³. This mechanism may also be relevant to the mouse α -globin pseudogene which lacks introns; it is not linked to the α -globin cluster²⁴ and has recently been found to be flanked by A-particle genes²⁵, which are retrovirus-like genetic elements. Finally, the amplification may represent a response to a functional lesion in p21 or to a relative resistance of these cells to the protein. However, we presume that the animals tolerate the amplification because these amplified genes are not ordinarily expressed, as suggested by the normal p21 levels in the *M. pahari* cell line, but firm conclusions must await further study of the expression and biological potential of these genes.

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DNA sequence homology and chromosomal deletion at a site of SV40 DNA integration

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The structure of simian virus 40 (SV40) DNA insertions is different from those of retrovirus proviruses and movable genetic elements. No single DNA sequence in either the cell or the SV40 genome serves as an obligatory site of SV40 integrative recombination^{1–9}, and SV40 DNA insertions are not bordered by the repeat structures characteristic of transposons and retrovirus proviruses^{10–15}. Integration of SV40 could involve the matching of short stretches of homologous sequences present in the otherwise heterologous SV40 and cellular genomes. To explore this possibility, I have now sequenced a rat DNA fragment that contains an unoccupied site of SV40 DNA integration. Comparison of this sequence with that of SV40 and that at the rat-SV40 recombinant junction allowed two conclusions: first, SV40 DNA became linked to rat DNA at a point where the two genomes shared 5 base pairs (bp) of DNA sequence homology; and second, a rearrangement of the rat genome, probably a deletion of at least 3 kilobases (kb), occurred at the site of SV40 integration.

The SVRE9 cells, chosen for this study, were Fisher rat embryo fibroblasts that were transformed by infection with SV40¹⁶. In previous work, the single viral DNA insertion in those cells was cloned and the sequences at the rat-SV40 junctions were determined⁸. Figure 1 shows the structures of the cloned SV40 DNA insertion, Sst9, and the subcloned SV40–rat junction fragments, 9M and 9L.

To test their suitability as probes with which to isolate a rat DNA fragment containing the unoccupied site of SV40 integration, junction fragments 9M and 9L were radiolabelled and hybridized to Southern¹⁷ blots of restriction endonuclease-digested rat DNA. 9M DNA was unsuitable in that it hybridized to many size species of endo:R:EcoRI-digested rat DNA, indicating that this junction fragment contained a sequence that was repeated many times in the rat genome (not shown). By contrast, 9L DNA hybridized to a single rat DNA fragment and was apparently free of repeated DNA sequences (see Fig. 2).

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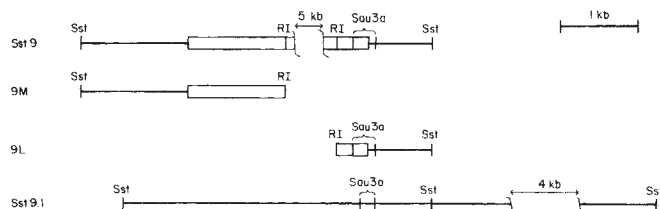


Fig. 1 Physical maps of cloned DNA fragments. Open bars represent SV40 DNA, lines represent cellular DNA. Sst9 was cloned from a λ phage library, 9M and 9L were cloned in plasmid pBR322. Cloning and analysis of this DNA were as previously described⁸. Clone Sst9.1 was isolated as described in the text and mapped by restriction endonuclease analysis and by hybridization using 9L DNA as a probe. The DNA fragments are drawn to align homologous sequences.

Figure 2 shows that SVRE9 cells contained two DNA fragments with homology to 9L DNA; one fragment co-migrated with the fragment bearing the SV40 DNA insertion, and a second fragment co-migrated with the normal rat DNA fragment homologous to 9L DNA. These data are most simply interpreted to indicate that SVRE9 cells are haploid for their single SV40 DNA insertion; the two SVRE9 DNA fragments detected by the 9L DNA probe probably originated from a pair of homologous chromosomes, one carrying the SV40 DNA insertion and the other containing a copy of the unoccupied SV40 integration site.

The putative unoccupied integration site was isolated from a library of endo:R:SstI-digested SVRE9 DNA using procedures previously described^{8,9}. The chimaeric λ phage Sst9.1 (shown in Fig. 1) contained two SstI fragments 4 kb and 6 kb in size. Analysis by restriction endonuclease digestion and blot hybridization (Fig. 3 and other data not shown) mapped the 9L homology to one end of the 4 kb fragment of Sst9.1 DNA, as indicated in Fig. 1.

To determine whether any of the DNA sequences flanking the 9M junction were present in Sst9.1 DNA, radiolabelled 9M DNA was hybridized to Southern blots of SstI-digested Sst9.1 DNA. As shown in Fig. 3, none of the DNA in Sst9.1 was homologous to 9M DNA. The absence of homology between 9M DNA and Sst9.1 DNA is most simply explained by deletion of at least 3 kb of the rat genome at the site of SV40-rat DNA linkage in the SVRE9 progenitor cell.

Analysis of Sst9.1 DNA by restriction endonuclease digestion and blot hybridization localized the SV40 integration site to a 200-bp endo:R:Sau3a fragment (see Fig. 1). The nucleotide sequence of this DNA fragment was determined by the protocol of Maxam and Gilbert¹⁸ and the results are shown in Fig. 4. Comparison of the Sst9.1 DNA sequence with those of SV40 and the 9L junction revealed that SV40 DNA and rat DNA shared a 5-bp sequence homology at a point coincident with the rat-SV40 junction in 9L DNA.

This is the first direct demonstration that DNA sequence homology may mediate integrative recombination between SV40 DNA and the cellular chromosome. Two other studies indicate that animal cells have the ability to recombine heterologous DNA molecules through short sequence homologies. Gutai¹⁹ found two cases of 3-bp homologies at virus-virus recombinant joints in naturally arising variants of SV40. M. Botchan and co-workers (personal communication) have found evidence that short sequence homologies are involved in the imprecise excision of a SV40 DNA insertion.

The DNA sequence of the unoccupied SV40 integration site shown in Fig. 4 is striking in two ways. It is repetitive, containing 12 T·G pairs in tandem, and it is composed almost entirely of G and T. Thus, this DNA chain is simple in both its sequence arrangement and its base composition. The T·G repeat does not extend past the nucleotides shown in Fig. 4. In addition to the 34 bases shown, the sequence 80 nucleotides to the left and 25 nucleotides to the right were determined. The T·G repeat is remarkably similar to the 'simple sequence' DNA

reported by Slightom *et al.*²⁰ to be present in the second introns of human γ -globin genes. Comparison of sequence similarities and differences between allelic γ genes and non-allelic γ and γ genes led these authors to suggest that this simple sequence region was a hot spot for DNA exchanges leading to gene conversion. As the non-allelic human γ -globin genes are ~97% homologous, simple sequence DNA is postulated to provide more than sequence homologies through which to initiate strand exchanges between genes. Rather, simple sequence DNA seems to act specifically by virtue of its unusual sequence, at least in the case of human γ -globin genes. Gene conversion has been suggested as a mechanism by which multi-gene families may conserve the identity of their members²¹, and simple sequence DNA may be of general importance in the gene conversion processes. The T·G repeat found in rat DNA may thus be one of many such repeats in the cellular genome.

These considerations bear on the evaluation of the possible contribution of the simple sequence DNA in Fig. 4 to the integration of SV40 DNA. There are two obvious possibilities: (1) simple sequence DNA may be a target for specialized recombination enzymes designed to accomplish gene conversions; these enzymes may act to integrate SV40 DNA. (2) Simple sequence DNA may be present in many copies, thereby increasing the probability of integration in such a sequence.

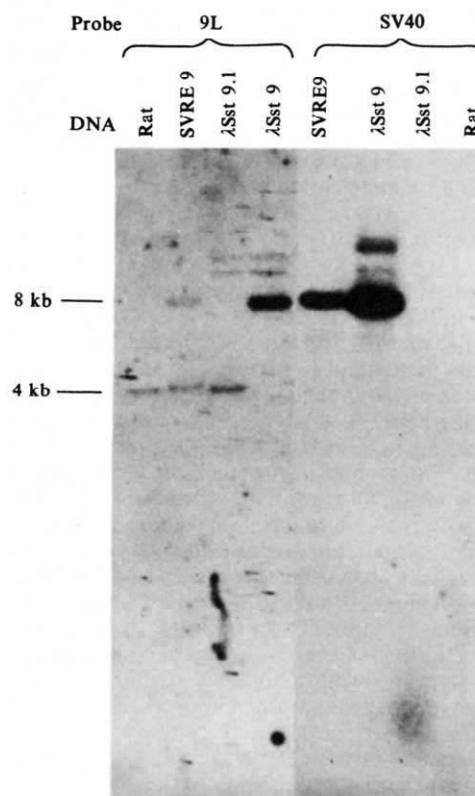


Fig. 2 Detection and sizing of rat DNA fragments containing sequences homologous either to SV40 DNA or to DNA flanking the 9L junction of the SV40 DNA integrated in SVRE9 cells. DNA (10 μ g) from either Fisher rat embryo cells or SVRE9 cells were digested with SstI and electrophoresed through 1% agarose in 36 mM Tris, 30 mM NaH₂PO₄, 1 mM EDTA pH 7.5. Chimaeric λ phage, Sst9 and Sst9.1, bearing the DNA fragments shown in Fig. 1, were mixed with calf thymus DNA and cut with SstI. 1×10^{-5} μ g of each phage DNA was run in parallel with rat cell DNA samples. By methods previously described¹, gels were blotted on to nitrocellulose which was then hybridized to either 9L DNA in pBR322 or SV40 DNA, each of which had been labelled to high specific activity by nick translation. DNA homologous to radioactive probes was detected by autoradiography. Faint bands in λ Sst9 and λ Sst9.1 tracks in the 9L panel are due to weak hybridization between pBR322 and λ arms. Minor bands in λ Sst9 track in SV40 panel are partially digested chimaeric phage DNA.

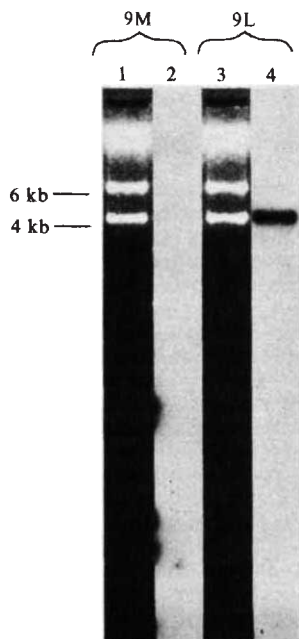


Fig. 3 Lack of homology between the rat DNA sequences in clone Sst9.1 and the rat DNA flanking the 9M junction of the SV40 DNA integrated in SVRE9 cells. A λ phage-bearing Sst9.1 DNA (Fig. 1) was digested with *Sst*I and electrophoresed on a 0.7% agarose gel. The gel was stained with ethidium bromide, photographed under UV illumination, blotted and the blot hybridized to one of two radiolabelled probes: 9M DNA, lanes 1 and 2; or 9L DNA, lanes 3 and 4. Lanes 1 and 3 are ethidium bromide-stained gels. Lanes 2 and 4 are autoradiographs of the same gels, blotted and hybridized as described elsewhere⁸. 9M DNA did not hybridize to either of the cell DNA fragments in Sst9.1, while 9L DNA hybridized strongly to the 4-kb DNA fragment present in Sst9.1

Although both possibilities are attractive, there is no reason to believe that simple sequence DNA is necessarily involved in integration of SV40 DNA. The sequence labelled 'SVRE9' in Fig. 4 shows that homologous recombination of SV40 DNA with a portion of the repetitive rat DNA sequence generated a SV40-cell junction in which the terminal viral sequence, TGTGT, is repeated in the adjacent cellular DNA. In previous analysis of the nucleotide sequences at other cell-virus junctions, I found a similar repeated structure, composed of C and T, present at one junction of the SV40 DNA insertion present in SVRE17 cells⁸. However, of the six cell-virus recombinant junctions compared in that study, only two, one junction of SVRE9 and one of the SVRE17 DNA insertion, showed repeat structures. In addition, none of the three viral DNA insertions compared in that study showed repeat structures at both ends of an integrated SV40 DNA. Therefore, although it is not uncommon, repeated, simple sequence DNA is not a requisite feature of DNA adjacent to SV40 DNA insertions. The data in Fig. 4 suggest that the presence of a terminal repeat structure at a cell-virus junction may be indicative of homologous integration into a repeated cellular sequence, but the lack of such structures does not rule out homologous recombination with non-simple sequence DNA.

Integration of SV40 DNA may exploit the great sequence complexity of the mammalian genome. Any 5-bp sequence present in the SV40 genome would be expected to occur of the order of 10^6 times in the unique sequence component of a rat cell genome. Perhaps integration is initiated when a random short sequence in the viral genome finds its match in chromosomal DNA. Integration might be completed by a second, independent ligation reaction at a different chromosomal location. Such a mechanism, of course, would generate chromosomal deletions at integration sites. Models predicting chromosomal deletions have been proposed previously (ref. 6 and

Botchan in ref. 22) and all evidence to date indicates that deletion of substantial amounts of the cellular chromosome is a consequence of SV40 DNA integration. The SVRE9 chromosome suffered a loss of at least 3 kbp of DNA, and there has been one other report consistent with chromosomal deletion at a SV40 DNA integration site⁹. Analysis of a third SV40 DNA insertion, in SVRE17 cells, also indicates a loss of chromosomal sequences at the site of SV40 DNA integration (J.R.S., unpublished data). Three examples do not compel the conclusion that SV40 DNA integration is always accompanied by chromosomal deletions, but no exceptions have yet been found.

The finding of short sequence homology at a SV40 DNA integration site implies that DNA sequence homology might direct an exogenous DNA to integrate homologously into a unique chromosomal copy of itself. This is the case in yeast cells; exogenous yeast genes recombine homologously with their chromosomal counterparts²³. However, there is reason to doubt the ability of animal cells to match an exogenous DNA sequence efficiently with a single chromosomal copy of the same sequence. Botchan *et al.*²⁴ reported that when SV40-transformed rat cells were superinfected with SV40, the newly introduced viral genomes did not integrate into pre-existing SV40 DNA insertions. The difference between yeast cells and rodent cells may simply reflect the larger size of the mammalian genome. Exogenous SV40 DNA may not be able to find a single chromosomal copy of itself, and instead be integrated through the mediation of a short, but highly frequent, random sequence homology. Whatever the reason, integration of exogenous DNA into a unique homologous chromosomal site of an animal cell genome seems to be a low probability event.

SV40 integration is the best studied case of incorporation of a foreign DNA molecule into the cellular genome. Many SV40 DNA insertions have been analysed by restriction endonuclease mapping¹⁻⁷ and six SV40-cell recombinant junctions have been sequenced^{8,9}. These studies have established that, unlike retroviruses and movable genetic elements, integration of SV40 DNA insertions does not involve specific DNA sequences in either the viral or cellular genomes, nor does it involve generation of short duplications of host sequence at the point of integration. Although analysis of more unoccupied sites of SV40 DNA insertion will be needed to understand fully the role of base pairing in SV40 integration, the data presented above demonstrate that cells are able to use a short sequence homology to integrate an otherwise heterologous SV40 DNA molecule. This example of homologous recombination suggests an additional difference between integration of SV40 DNA and retroviruses and transposition of movable elements.

Understanding cellular recombination systems that foster integration of exogenous DNA is important in assessing the practicability of schemes that propose the introduction of therapeutic genetic material into the genomes of defective cells. Integration of SV40 DNA probably reflects the action of a cellular recombination system that can incorporate any foreign



Fig. 4 DNA sequences at the SV40 integration site. The sequence in Sst9.1 DNA homologous to the rat DNA immediately adjacent to SV40 DNA in clone 9L was shown, by hybridization experiments, to lie in a 200-bp *Sau*3a fragment (see Fig. 1). The nucleotide sequence of 140 bp of this DNA was determined by the method of Maxam and Gilbert¹⁸, and the relevant portion of the sequence is labelled 'Rat'. Also shown is the sequence of SV40 DNA as reported by Reddy *et al.*²⁵, and the sequence at the 9L junction of the SV40 DNA insertion in SVRE9 cells, as previously determined by J.R.S.⁸. Vertical lines designate homologous nucleotides. Comparison shows that SV40 DNA and rat DNA share 5 bp of homology at a point coincident with the SV40-rat junction in the cloned 9L junction fragment.

DNA into the cellular genome, and analysis of SV40 DNA insertions may predict the fate of other exogenous DNAs. Although cells may incorporate exogenous DNA efficiently, that DNA is unlikely to integrate into a homologous single-copy sequence in chromosomal DNA. Instead, foreign DNA might be expected to integrate randomly through a mechanism that uses short sequence homologies, and generates chromosomal deletions.

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Influenza virus RNA is synthesized at fixed sites in the nucleus

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We have recently shown that cellular RNA is synthesized at a sub-nuclear structure, the nuclear cage¹, which contains proteins also found in other structures called variously the nuclear pore complex, lamina, envelope and matrix²⁻⁶. Is the RNA of an exogenous virus also synthesized at the cage? We chose to study influenza virus as it is unusual in its requirement for a host cell nucleus even though there are no cellular counterparts to the transcription of the infecting negative strands of genomic influenza RNA, nor to the replication of the resulting positive RNA strands to form new virion RNA. The cellular sites of these processes have not yet been definitively demonstrated^{7,8}. We now show that nascent viral transcripts are closely associated with the cage and we conclude not only that transcription and replication of viral RNA are nuclear, but also that they occur at fixed sites in the nucleus.

Several observations suggest a nuclear involvement during the transcription and replication of influenza virus⁷⁻¹⁰. These include nuclear labelling in autoradiographs, inhibition of viral production by actinomycin D and α -amanitin—both inhibitors of nuclear RNA polymerase II—and the splicing and methylation of viral mRNA. However, attempts to demonstrate directly the site of viral RNA synthesis by pulse-labelling and cell fractionation have yielded variable results¹¹⁻¹³. We believe that this variability is caused by long pulse-labels (≥ 30 min) and nucleolytic action during cell fractionation, both of which permit

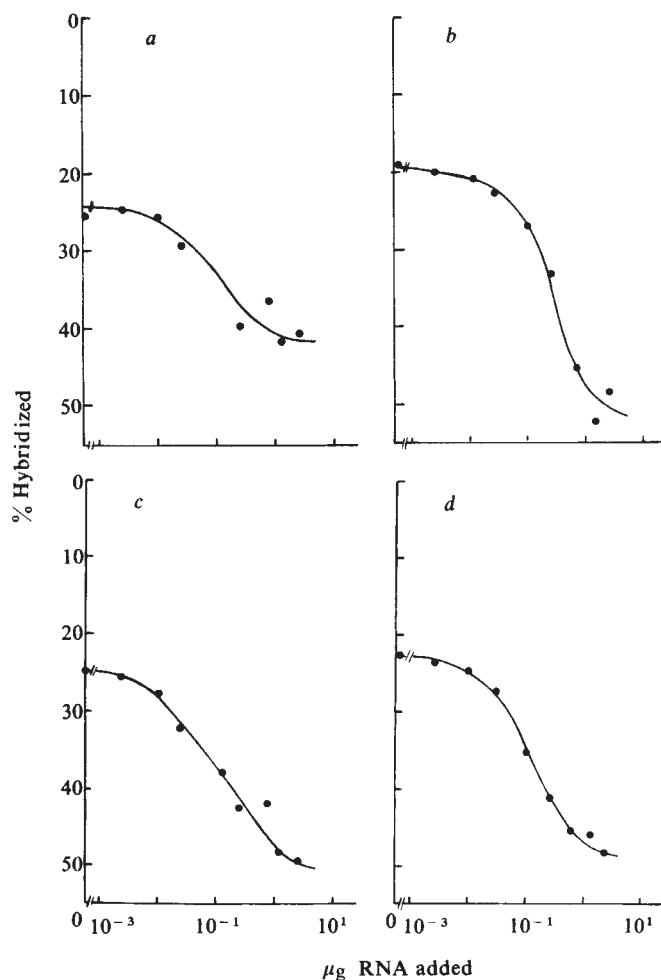


Fig. 1 The proportion of viral transcripts in pulse-labelled RNA from infected cells. Cells were infected and pulse-labelled (2.5 min) with ³H-uridine after 2.5 h (a, b) or 4.5 h (c, d). Nucleoids were then isolated as described in Table 1 legend, pulse-labelled RNA was purified and hybridized¹ with various amounts of virus-specific RNA obtained from purified virions (vRNA) or by extracting polyadenylated RNA from the cytoplasm of infected cells 7 h post-infection (mRNA)¹⁸. (Such a mRNA preparation is impure and contains some cellular RNA, and perhaps low levels of vRNA. However, the cellular impurities have no complements and so cannot form hybrids.) Less than 5% of pulse-labelled RNA from uninfected cells hybridized with these virus-specific RNAs. The per cent forming a hybrid resistant to S₁ nuclease was determined^{1,31}. Hybridizations (10 days) were carried out in a volume of 5 µl containing at least 5,000 c.p.m. of pulse-labelled RNA (~2.5 µg of total nucleoid RNA), 2.5 µg tRNA and up to 2.5 µg mRNA (a, c) or vRNA (b, d). These conditions give a maximum C₀t of 10³ mol s l⁻¹.

transfer between cell compartments. We have therefore used very short pulses (2.5 min) and a cell fractionation procedure which minimizes nucleolytic degradation.

Embryonic chick fibroblasts were lysed in a non-ionic detergent, 2 M salt and a chelating agent. The resulting nucleoids were sedimented free of cytoplasmic material and contained naked histone-free DNA packaged within a flexible cage of RNA and protein¹⁴⁻¹⁷. Their DNA, which is looped by attachment to the cage, is supercoiled^{14,15}, indicating that nucleolytic degradation has been suppressed during isolation.

When mock-infected fibroblasts are incubated with ³H-uridine for 2.5 min, 96% of the cellular radioactivity which is insoluble in trichloroacetic acid subsequently co-sediments with nucleoids (Table 1). Nearly all the label also co-sediments with nucleoids made from infected fibroblasts pulsed at early (2.5 h) or late (4.5 h) times during infection. We determined what proportion of nascent RNA in infected cells was virally coded

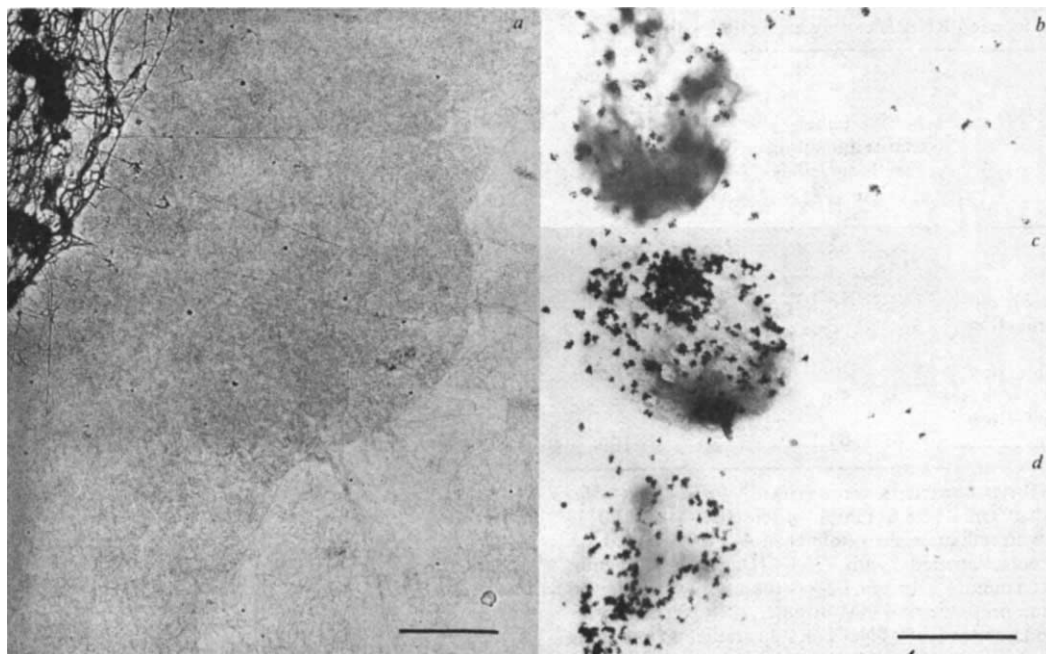


Fig. 2 Electron micrographs of nucleoids spread by Kleinschmidt's procedure. *a*, A typical spread illustrating part of the cage and skirt of tangled DNA fibres, which extend to the edge of the field. *b-d*, Autoradiographs of spreads¹⁹ after labelling uninfected fibroblasts for *b*, 24 h with ³H-thymidine (0.01 μ Ci ml⁻¹; 58 Ci mmol⁻¹) or for *c*, 2.5 min with [5-6-³H]uridine and (2,5'-8-³H]adenosine (both at 100 μ Ci ml⁻¹ and ~40 Ci mmol⁻¹); or *d*, infected fibroblasts 4.5 h post infection for 2.5 min with ³H-uridine and ³H-adenosine as in *c*. So that silver grains can be easily counted in *b-d*, DNA is shadowed but not stained and can be seen spread to the edge of the field only at higher magnifications. In *b*, grains lie over both cage and skirt, whereas in *c* and *d* they lie predominantly over the cage. Conditions for infection are described in Table 1 legend. The bars in *a* and *d* represent 5 and 2.5 μ m respectively. *b-d* Are at the same magnification.

by isolating pulse-labelled nucleoid RNA and hybridizing it with an excess of unlabelled virion (v) RNA or mRNA (Fig. 1), which hybridize with strands of positive and negative polarity respectively¹⁸. In the absence of added vRNA or mRNA, <1% of the pulse-labelled RNA from mock-infected cells self-anneals, reflecting the asymmetric transcription of cellular DNA. In contrast, about 25% from infected cells self-anneals, reflecting the synthesis of both positive and negative strands. In the presence of an excess of either vRNA or mRNA, 45–50% of the pulse-labelled RNA hybridizes, whereas ~70% hybridizes in the presence of an excess of both vRNA and mRNA (results not shown). We cannot give a precise estimate of the proportion of nascent transcripts which are viral, as our mRNA probe is impure (see Fig. 1 legend) and the significance of the self-annealed values is difficult to assess. Nonetheless, it is clear that positive and negative viral transcripts constitute a significant proportion (>50%) of the pulse-labelled material.

The pulse-label might co-sediment with cages, either because it is specifically attached or because it is tangled in the high concentration of nuclear DNA. We tested the latter possibility by finding out whether RNA could be released on detaching DNA from the cages. Cells were labelled with ¹⁴C-thymidine for 24 h to label their DNA uniformly, then infected and subsequently pulsed with ³H-uridine. Nucleoids were isolated, incubated with the restriction endonuclease, *Eco*RI, the cages were filtered free of detached DNA and the amounts of the two labels remaining associated with them determined¹⁹ (Table 1). When >75% of the ¹⁴C (that is, DNA) was detached, <1% of the ³H (RNA) was lost, showing that the pulse-labelled RNA cannot be detached with DNA from the cages.

A second experiment confirms that nascent RNA is not simply entangled in DNA or the cage. When nucleoids are spread on an air-aqueous interface, their DNA, initially confined within the cage, spreads out from the cage to form a surrounding skirt of tangled DNA fibres¹⁷ (Fig. 2*a*). Cages from chick fibroblasts are not as robust as those of the HeLa nucleoids that we have studied extensively, so the DNA is less protected

from shear. As a result, more is broken and therefore appears relaxed after spreading. The spread DNA is less dense, reflecting the lower DNA content of the diploid chick nucleus. The distribution of DNA in skirt and cage was obtained by reference to autoradiographs of spreads prepared from cells containing uniformly labelled DNA (Fig. 2*b*); 53% (average of 15 spreads) of the DNA is outside the cage. However, when cells—whether infected or not—are pulse-labelled with ³H-uridine for 2.5 min, >85% of the grains lie over the cage in each of 10 spreads selected at random (Fig. 2*c, d*). The proportion of grains lying over the nucleolus is reduced in spreads from infected cells (see Fig. 2*c, d*) late in infection, reflecting the reduced percentage of ribosomal (cellular) RNA synthesis in the total. The nascent RNA—largely viral—is unable to escape with the DNA from the cage.

A trivial explanation consistent with these results is that nascent viral RNA, synthesized throughout the nucleus, might stick nonspecifically when nucleoids are prepared. Our earlier study¹ showed that nascent RNA in uninfected HeLa cells was specifically attached at the 5' end, whereas added RNA, or RNA synthesized *in vitro*, was either unattached or was nonspecifically associated with cages but could be detached from them by *Eco*RI or spreading. Using identical conditions we have shown here that nascent viral RNA is inseparable from cages. Three further controls make a nonspecific association even less likely. First, when pulse-labelled RNA from infected cells is purified and mixed with unlabelled infected cells immediately before lysis, <3% of the pure RNA co-sediments with the nucleoids, indicating that the added RNA has little affinity for cages. Second, we have confirmed earlier results⁹ which showed by autoradiography that all the pulse-labelled RNA in infected cells was nuclear. After a 2-h chase, the total number of grains over the cell was reduced, reflecting turnover: half lay over the nucleus, the remainder being cytoplasmic (results, not shown, of experiments using conditions described in Table 1). When these cells are lysed in 2 M salt, only 40% of the label now co-sediments with the nucleoids (Table 1). Although

Table 1 Pulse-labelled RNA is closely associated with cages

	% Label co-sedimenting with nucleoids	% Label remaining associated with cages after incubation with <i>EcoRI</i>	
		¹⁴ C	³ H
Mock-infected cells (2.5 min pulse)	96	17	100
Mock-infected cells (2.5 min pulse, 2 h chase)	58	—	—
Cells 2.5 h post-infection (2.5 min pulse)	93	25	99
Cells 2.5 h post-infection (2.5 min pulse, 2 h chase)	40	—	—
Cells 4.5 h post-infection (2.5 min pulse)	92	20	100

Primary chick embryo fibroblasts were grown³⁰ for 24 h in [¹⁴C]thymidine (0.01 μ Ci ml⁻¹; 58 Ci mmol⁻¹), infected (MOI 100:1) where appropriate with influenza virus (influenza A/PR8/34) and 2.5 or 4.5 h later pulse-labelled with [5,6-³H]uridine (2.5 min; 100 μ Ci ml⁻¹; ~40 Ci mmol⁻¹). In some cases the pulse was followed by a 2-h chase in the presence of 1 mM uridine. 2.5×10^6 cells were removed, washed and lysed in 1.95 M NaCl on step gradients containing 1.95 M NaCl, spun (Beckman SW50.1 rotor; 15,000 r.p.m.; 45 min) to sediment the nucleoids on to the step and the percentage of ³H co-sedimenting with the nucleoids was determined^{15,16}. Nucleoids were collected, diluted to 0.2 M NaCl, incubated with *EcoRI* and the proportion of labels remaining associated with cages was determined after filtration^{1,19}. A control experiment showed that >97% of the ³H but none of the ¹⁴C could be detached from cages by ribonuclease.

nucleoids contain some elements of the cytoskeleton (that is, actin and intermediate filaments), the cytoplasmic RNA does not co-sediment with the nucleoids.

For the third control we studied Chandipura virus, a rhabdovirus with a single genomic RNA strand of negative polarity and whose reproduction is cytoplasmic²⁰. Plaque-purified virus²¹ was grown in chick fibroblasts (conditions and multiplicity of infection similar to those described in Table 1 legend so that cytopathic effects were visible after 7 h) in the presence of a concentration of actinomycin D (0.5 μ g ml⁻¹ added 1 h post-infection) which suppresses nuclear RNA synthesis by >95%. When, at 4 h post-infection, cells were labelled for 10 min with ³H-uridine (50 μ Ci ml⁻¹), about 3.7 times more label was incorporated into infected cells than into mock-infected controls; thus, 73% of the labelled transcripts were viral, the remainder being the residual host transcripts. When such cells are lysed and spun, only 30% of the incorporated label co-sedimented with nucleoids; 70% was cytoplasmic and remained at the top of the gradient. Unlike nascent influenza virus transcripts, the transcripts of Chandipura virus are not associated with cages.

We have demonstrated that at early and late times during infection with influenza virus, at least 50% of the nascent transcripts in infected cells are viral and all are attached to the nuclear cage. They remain attached in the detergent and 2 M NaCl used to prepare nucleoids. We might have expected little host involvement in RNA-dependent RNA synthesis, especially as the infecting virus contains a polymerase which is capable of synthesizing its own mRNA *in vitro*^{22,23}. It is perhaps not surprising that nascent transcripts of positive polarity are cage associated because they are covalently coupled at their 5' ends to host-encoded sequences^{24,25}, which we have shown are themselves attached¹. Attachment of nascent transcripts of negative polarity, which do not contain any host-encoded sequences, is more surprising. However, a complex picture of processing of nuclear transcripts is now emerging. Transcripts are attached to the nuclear cage as they are generated¹ and subsequently as heterogeneous nuclear RNA²⁶⁻²⁸; later they are attached to the cytoskeleton as cytoplasmic message²⁹. (This cytoskeletal attachment is unstable in high salt concentrations.) There are

obvious practical advantages in tying a long RNA molecule to a larger structure during such a complex series of events as synthesis, capping, methylation, splicing, polyadenylation and translocation to the cytoplasm. There are additional problems associated with the regulated transcription and replication of the eight influenza viral segments and attachment could provide a structural basis for this. Therefore, attachment may play a central part in these processes and any viral nucleic acid subverting some, or all, of them, must also become attached.

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Cytogenetic location and expression of collagen-like genes in *Drosophila*

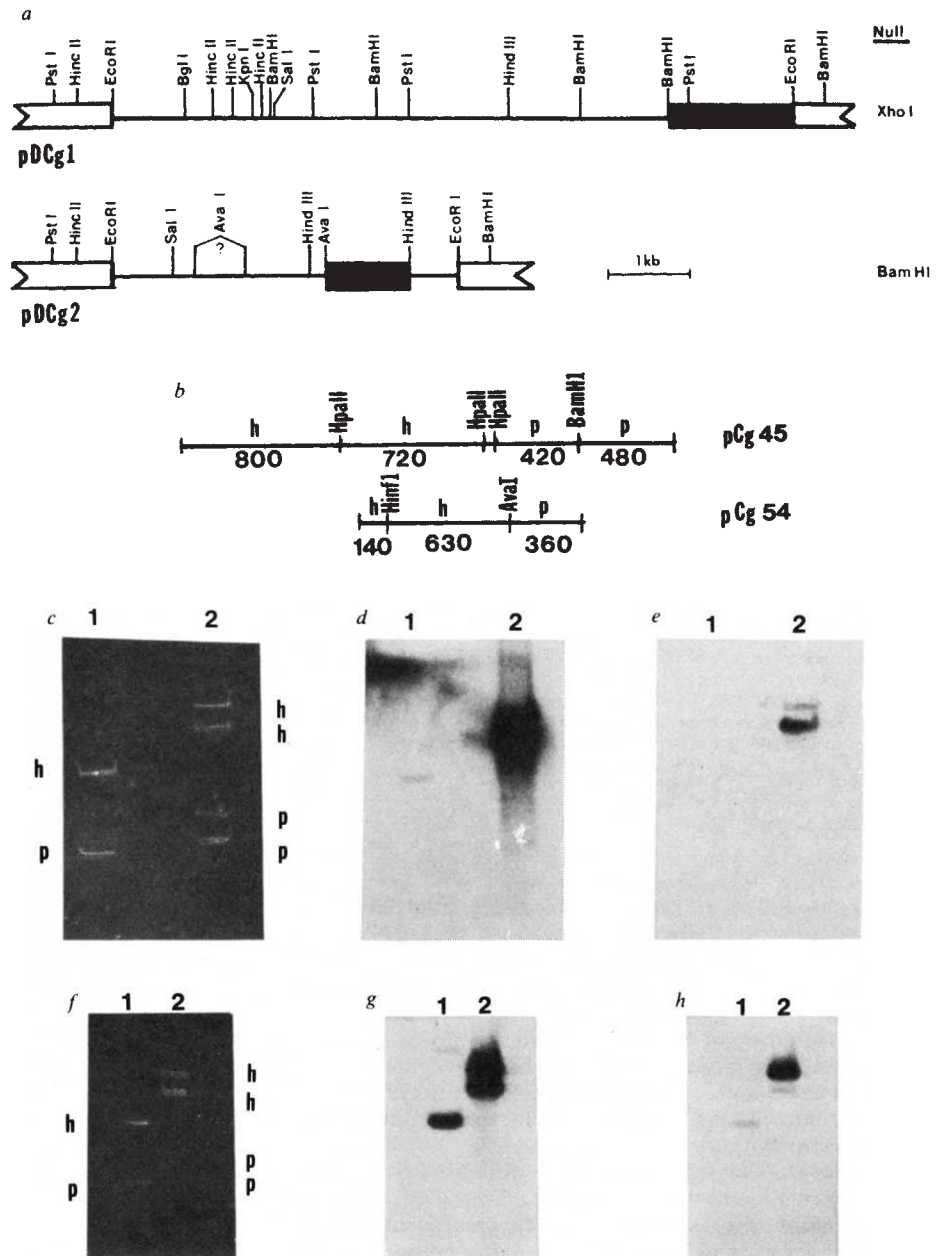
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Much of the present interest in vertebrate collagens stems from the important part which these extracellular, structural proteins play in developmental processes and tissue organization as well as from their complex gene structure. So far the only vertebrate collagen genes examined encode the constituent polypeptide (pro α) chains of type I procollagen, that is, the pro $\alpha 2(I)$ genes from chicken^{1,2} and sheep³, and the pro $\alpha 1(I)$ gene from mouse⁴. Recently, we have isolated several collagen-like genomic DNA clones from *Drosophila melanogaster*⁵. In addition to providing data on the evolutionary history of this gene family, studying *Drosophila* has distinct advantages for cytogenetic localization of genes and for defining the functional roles of individual collagens by the application of genetic techniques. Here we compare the hybridization patterns, cytogenetic localization and expression of two of the *Drosophila* clones, DCg1 and DCg2. Although they are cytogenetically unlinked, they share similar developmental RNA profiles.

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Fig. 1 Radioactive probes constructed from the *Drosophila* sequences DCg1 and DCg2 were hybridized to restriction digests of chicken collagen cDNA sequences to determine the regions of homology between the *Drosophila* and chicken sequences. **a**, Restriction maps of DCg1 and DCg2. Open boxes represent pBR322 vector sequences flanking the inserted *Drosophila* DNA and solid boxes represent regions of *Drosophila* DNA that cross-hybridize to the chicken clone, pCg45. Radioactive probes representing the solid regions were made by ^{32}P nick-translation²⁶ of the following restriction fragments purified from agarose gels by electroelution: pDCg1, 1.5-kb *EcoRI*/*Bam*HI fragment; pDCg2, 1.2-kb internal *Hind*III fragment. **b**, Digestion with the appropriate restriction enzymes divides the chick collagen cDNA insert sequences into discrete fragments that encode either the Gly-X-Y repeat involved in triple helix formation (h) or the carboxy-terminal propeptide region (p) of the pro α -chain^{6,7}. The pro α 2 clone (pCg45) can be cut with *Hpa*II and *Bam*HI to produce two fragments of 800 and 720 base pairs (bp) encoding regions participating in triple helix formation, and two smaller fragments of 480 and 420 bp representing the propeptide region (see lane 2 of **c** and **f**). The pro α 1 clone (pCg54) can be cut by *Ava*I and *Hinf*I into fragments of 630 and 140 bp involved in triple helix formation and a fragment (360 bp) encoding propeptide sequences (see lane 1 of **c** and **f**). **c-h**, The *Drosophila* probes were hybridized to Southern blots of the restricted chick pro α 1 and pro α 2 collagen cDNA sequences to determine whether the shared homology was localized in areas encoding the triple-helix forming (h) or propeptide (p) regions of the chick sequence. **c-e**, The DCg2 probe was hybridized to filters in a 50% formamide, $5 \times \text{SSC}$ hybridization solution²⁶, and washed at 50°C in $0.5 \times \text{SSC}$, 0.1% SDS; a long exposure (**d**) and shorter exposure (**e**) of the autoradiograph are shown. **f-h**, The DCg1 probe was hybridized as above and washed first at 50°C in $0.5 \times \text{SSC}$ (**g**) and then at 50°C in $0.1 \times \text{SSC}$ (**h**). The DCg2 probe hybridizes preferentially to one of the fragments (720 bp) encoding the triple-helix forming (h) region of Cg45 while the DCg1 probe hybridizes most strongly to the other fragment (800 bp) that encodes the triple helix-forming region.



The differences in hybridization of each *Drosophila* clone to two chicken collagen cDNA clones suggests that the *Drosophila* sequences represent different members of a family of related collagen-like genes. The isolation of these *Drosophila* clones was based on sequence homology with a heterologous hybridization probe, the cDNA clone pCg45, encoding half of the chicken pro α 2(I) chain⁶. As shown in the restriction maps for the subclones pDCg1 and pDCg2 (Fig. 1a), the region of homology to Cg45 extends over only a portion of each clone. Hybridization of Cg45 to these designated subfragments is limited to the region of the cDNA clone encoding the Gly-X-Y sequences that participate in triple helix formation (the α -chain domain) (Fig. 1c-e). Each of the two *Drosophila* clones hybridizes preferentially to a different section of the chicken cDNA clone encoding the Gly-X-Y repeat (Fig. 1c-h). In addition, DCg1 shares more homology than DCg2 with the α -chain domain specified by a chicken pro α 1(I) cDNA clone⁷ (Fig. 1c-h). Although the number of genetically distinct collagen chains in *Drosophila* is unknown, there is biochemical evidence in other invertebrates for multiple chain types⁸⁻¹⁵. Therefore the differences in hybridization of the *Drosophila* clones to the chicken pro α 1(I) and pro α 2(I) cDNA clones suggest that the

former represent structurally distinct collagen-like molecules. However, if the two sequences use different codons preferentially, as observed for other collagen genes^{4,16}, they may simply represent non-allelic copies of a gene encoding one collagen-like protein. DNA sequence analysis of DCg1 indicates that it may encode a segment of a non-fibrous collagen such as a basement membrane or cuticle collagen, or a novel collagenous protein⁵.

At moderately low stringency, the genomic hybridization patterns defined for *Drosophila* embryonic DNA by the chick sequence Cg45 and the two *Drosophila* sequences DCg1 and DCg2 are each composed of several cross-reacting species (Fig. 2). Although the three patterns are not identical, it is clear that they do overlap. The similarities are more apparent between Cg45 and DCg1 than between Cg45 and DCg2, supporting the idea that the *Drosophila* sequences may encode proteins of two different structural classes. The 1.2-kilobase (kb) internal *Hind*III fragment of pDCg2 (see Fig. 1) used as a probe in this experiment hybridizes to a single *Eco*RI genomic restriction fragment, but labels two *Hind*III genomic restriction fragments with approximately half the intensity of the *Eco*RI hybridization band. These data indicate that there are two closely related

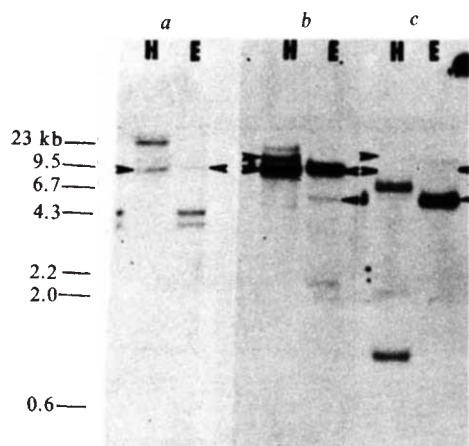


Fig. 2 Genomic hybridization patterns generated by Cg45, DCg1 and DCg2 sequences. Genomic DNA from *Drosophila* Canton S strain embryos (0–24 h) was digested to completion with either *Hind*III (H) or *Eco*RI (E), fractionated on 1% agarose gels and transferred to nitrocellulose filters²⁷. Each lane contained 5 µg DNA. ³²P-labelled probes were made from the cDNA insert of pCg45 by labelling with T4 DNA polymerase (Bethesda)²⁸ and from the DCg1 and DCg2 restriction fragments described in Fig. 1 by nick-translation²⁶. *a*, 1×10^6 c.p.m. of Cg45 probe were hybridized at 37 °C (ref. 26) in the presence of 10% dextran sulphate²⁹ for 24 h. The filter was washed in $0.5 \times$ SSC at 50 °C and autoradiographed for 4.5 days. *Drosophila* probes (2×10^6 c.p.m.) from DCg1 (*b*) or DCg2 (*c*) were hybridized at 37 °C as in *a*, washed at 50 °C in $0.1 \times$ SSC, and autoradiographed for 2 days. The mobilities of *Hind*III-restricted λ phage DNA used as size markers are indicated. Some shared cross-reacting sequences are indicated by arrowheads.

sequences located on the same genomic *Eco*RI fragment, which may explain the size discrepancy between the *Eco*RI band present in both the phage isolate and our subclone (4.3 kb) and the *Eco*RI band labelled in the genomic hybridization experiment (5.6 kb). During cloning and propagation in the λ vector, a recombination event between the two regions of homology may have deleted a portion of the original genomic *Eco*RI fragment. Alternatively, an artificial *Eco*RI site may have been introduced into a larger genomic *Eco*RI fragment during the λ library construction.

The same *Drosophila* sequences that were used as probes in the above experiments were hybridized *in situ* to salivary gland polytene chromosomes. These experiments identified the cytogenetic locus of pDCg1 as 25C on chromosome 2L and the locus of pDCg2 as 19EF/20AB near the proximal end of the X chromosome (Fig. 3). Thus the two *Drosophila* sequences are located at separate, unique cytogenetic sites and are not part of one complex gene locus. The DCg2 sequence is located within or near a probable heterochromatin/euchromatin boundary. Other workers^{17,18} have described active genes in analogous locations. The 25C locus for DCg1 is near a puff site in the 25AC region described by Ashburner¹⁹. This puff regresses in third instar larvae as ecdysone levels increase. This pattern of puffing is consistent with the developmental RNA profile of DCg1 described below. However, these similarities may be coincidental.

The accumulation of RNA transcripts homologous to the two *Drosophila* clones was measured in RNA populations representing sequential periods of the *Drosophila* life cycle (Fig. 4). A dot-blotting procedure was used in which aliquots of total RNA were immobilized on nitrocellulose filters and hybridized in probe excess (J.E.N. and B.J.M., unpublished observations). The maximal accumulation of homologous RNAs occurs during the first and second larval instars for both DCg1 and DCg2. RNA homologous to DCg1 is first detected during embryogenesis 12–15 h after oviposition, slightly preceding the first DCg2 hybridization signal. The presence of DCg2 transcripts extends beyond those of DCg1 into the third larval instar. RNA from cultured *Drosophila* Kc cells apparently contains transcripts

homologous to both DCg1 and DCg2. The hybridization and washing stringencies of these experiments demanded more precise base pairing than did the conditions used for the genomic blots in Fig. 2, and no significant cross-reaction occurred between DCg1 and DCg2 as measured by the DNA dots on each filter. The accumulation of DCg1 and DCg2 transcripts is clearly under developmental control.

Ultrastructural, biochemical^{9,11,20} and genetic²¹ studies in insects have demonstrated the existence of a basement membrane around internal organs in larval and adult stages, between the epithelium and muscle layer in larvae and also enveloping the imaginal disks. The developmental RNA profile that we have determined for the *Drosophila* sequences is consistent with the observed time courses for some of these processes, such as the initiation of internal or epithelial basement membrane formation during embryogenesis and early larval development. The pattern is equally consistent with larval cuticle deposition, although collagen-like proteins have not been reported to be components of this larval structure²². These

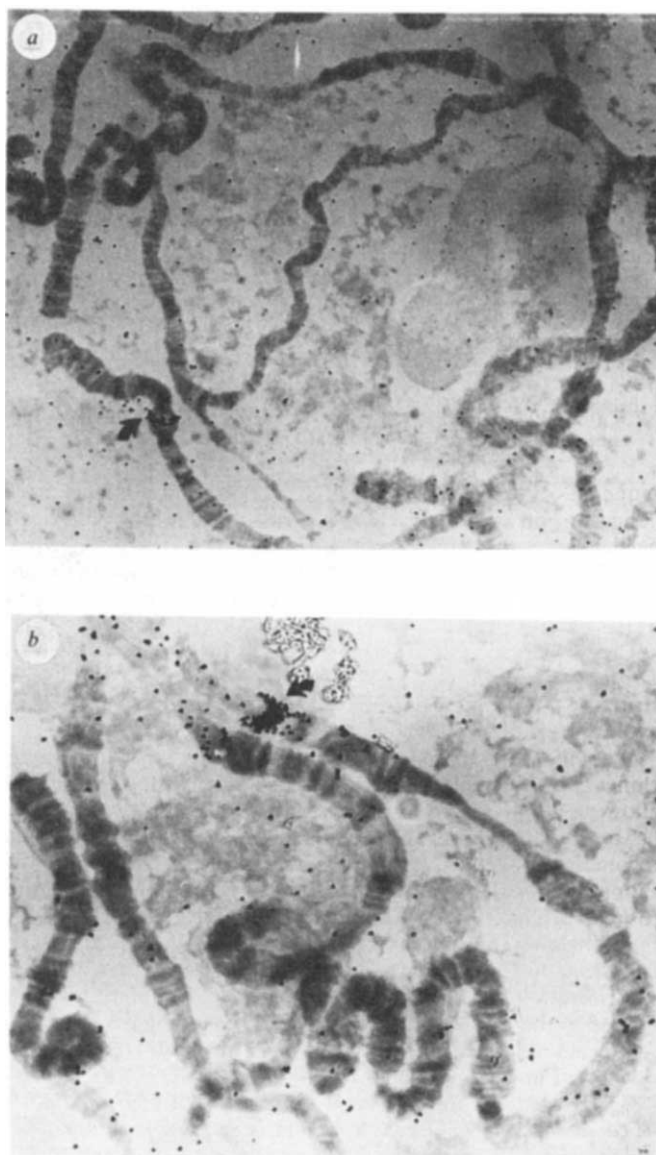


Fig. 3 *In situ* hybridization of DCg1 and DCg2 to *Drosophila* polytene chromosomes. ³H-cRNA probes were transcribed *in vitro* from DCg1 and DCg2 and hybridized at 60 °C to polytene chromosome squashes²⁶. Each slide received 4×10^5 c.p.m.; 14-day exposures. *a*, DCg1 hybridization over 25C on chromosome arm 2L; *b*, DCg2 hybridization over 19EF/20AB near the proximal heterochromatic region of the X chromosome. $\times 300$.

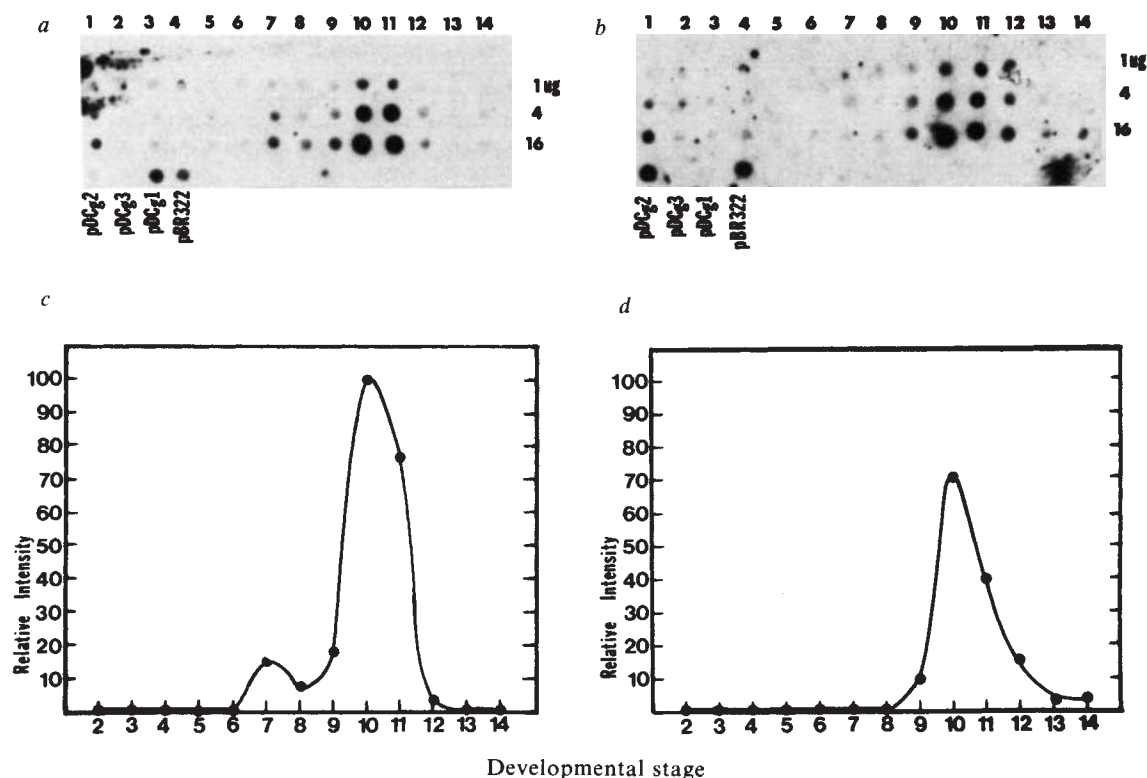


Fig. 4 Developmental mRNA profile of DCg1 and DCg2 expression. Total RNA was prepared from different *Drosophila* developmental stages³⁰ and immobilized on to nitrocellulose paper by loading aliquots in 20×SSC from a capillary pipette³¹. Three RNA concentrations (1, 4 and 16 µg) were used for each developmental stage. Each filter also contained control dots of the plasmids containing the *Drosophila* collagen-like sequences. Filters were hybridized by applying sufficient probe sequences to generate a local 10-fold sequence excess over the highest estimated target sequence concentration in any dot (J.E.N. and B.J.M., unpublished results). Hybridizations were done at 42 °C in 50% formamide, 5×SSC hybridization solution²⁶ and the filters were washed in an equivalent solution at 50 °C after 24–30 h hybridization. The figure shows autoradiographs of RNA dot blots hybridized with DCg1 (a) or DCg2 (b) sequences. c, d, The autoradiographs were quantitated using an LKB soft laser scanning densitometer. The maximum absorbance was calibrated for the most intense hybridization signal of DCg1 and other signals are expressed relative to that signal. The developmental stages represented are: 1, Kc cells; 2–9, periods during embryogenesis following oviposition by 0–2, 2–3, 3–6, 6–9, 9–12, 12–15, 15–18 and 18–21 h, respectively. 10–12, First, second and third instar larvae, respectively. 13, White prepupae. 14, Mid-pupation. An identical filter hybridized with pBR322 vector sequences showed hybridization only to control dots (data not shown).

sequences may also represent proteins containing a small, triple-helical region such as Clq²³ or acetylcholinesterase²⁴, although partial sequence data make this possibility unlikely for DCg1 (ref. 5). Neither pDCg1 nor pDCg2 show evidence of *in situ* hybridization to the *Drosophila* acetylcholinesterase locus at 87E1-5 (ref. 25).

Characterization of collagen genes in invertebrates such as *Drosophila* will hopefully allow genetic analysis of collagen structure and expression as well as providing insights into evolutionary relationships of vertebrate collagens.

We thank H. Boedtker for providing the chicken cDNA clones pCg45 and pCg54; J. Fristrom and T. Kornberg for allowing us to use their fly facilities for collection of embryos, larvae and pupae; and T. Maniatis for providing the *Drosophila* recombinant λ phage library used in screening. This work was supported by an NIH training grant to J.E.N. and an NIH grant to J.M.M. and B.J.M.

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Errata

In the letter 'Chorus-related electrostatic bursts in the Earth's outer magnetosphere' by L. A. Reinleitner, D. A. Gurnett and D. L. Gallagher, *Nature* **295**, 46–48 (1982), in Fig. 1 legend the words 'features of the chorus related electrostatic bursts. The slight inter-' have been repeated. Thus the second sentence should read 'The slight interference from 1738:45 to 1739:17 UT (also in Fig. 3) is from the ISEE electron density experiment.

In the article 'Protochordate allorecognition is controlled by a MHC-like gene system' by V. L. Scofield, J. M. Schlumpberger, L. A. West and I. L. Weissman, *Nature* **295**, 499–502 (1982), in Table 4 legend the percentage fusible progeny yielded by crosses where no alleles are shared should be 75%, not 7.5%.

In the letter 'Stereochemistry of iron in deoxyhaemoglobin' by M. F. Perutz, S. Samar Hasnain, P. J. Duke, J. L. Sessler and J. E. Hahn, *Nature* **295**, 535–538 (1982), in the final paragraph of page 537, references 14 and 12 should read 15 and 11, respectively. On page 538 the reference cited at the top of the second column of text should be 12, not 13.

MATTERS ARISING

The ν_3 fundamental band of the methyl radical

IN discussing near-IR absorption bands in IRS7, Allen and Wickramasinghe¹ remark that "laboratory data are not available for even quite simple, incompletely bonded molecules (for example, CH_3) such as might be found in the interstellar environment".

We have recently performed a laboratory measurement of the ν_3 fundamental band ($\nu_0 = 3,160.820 \text{ cm}^{-1}$) of CH_3 in absorption using a difference-frequency laser². The most prominent feature in the spectrum is the ${}^1Q_0(N)$ ($N = 2, 4, 6, \dots$) sub-branch. The transition frequency of the strongest line, ${}^1Q_0(2)$, is $3,154.7459 \text{ cm}^{-1}$ ($= 3.16983 \mu\text{m}$). Other strong lines at (300 K) are calculated to be: 3,101.045, 3,128.548, 3,199.730, 3,217.754, and $3,224.396 \text{ cm}^{-1}$. However, none of the prominent features reported by Allen and Wickramasinghe seem to fit our observed transition frequencies.

The methyl radical is expected to be an important species in certain interstellar sources³. In particular, estimates of the abundance of CH_3 in the atmosphere of the nearby carbon star IRC +10°216 indicate that its abundance relative to CO is $\sim 10^{-4}$ (ref. 4 and personal communications from E. M. McCabe, R. C. Smith and R. E. S. Clegg, and A. Kinney). Thus the methyl radical should be detectable in this source and we plan to conduct an IR search for it in the very near future.

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Saturn's rotation period

CARR ET AL.¹ have inferred Saturn's rotation period from data recorded by the Voyager-1 Planetary Radio Astronomy (PRA) instrument. They constructed profiles of the received power in the coordinate frame of the Saturn longitude system (SLS) determined by ourselves². After comparing profiles made from month-long spans taken ~ 7 months apart, Carr et al. attributed a longitude shift to a 2-s error in the SLS rotation period.

Some problems in the work of Carr et al. affect the validity of their result. First is the precision of their method. From basic statistics we would expect the standard deviation in the individual rotation period determinations to scale with the square root of the number of samples. Thus, as Carr et al. used 2 months of data whereas we used 8 months, the former's individual standard deviation should be no better than root (8/2) times that of the latter, or ± 14 s. In addition, this value should vary with observing frequency owing to the change in emission occurrence probability with frequency. The rotation period at 500 kHz cannot be determined nearly as well as at 150 kHz because the occurrence probability is at least a factor of 4 lower at 500 kHz (ref. 3).

Using PRA data, we attempted to assess the inherent uncertainty in their method. We compared flux density longitude profiles generated over different time intervals, but at the same frequency. For example, comparing the longitude profile made at 346.8 kHz from data only 1 month apart, we found a 70° shift, which would correspond to an uncertainty of 15 s over the ~ 7 month time span used by Carr et al. We repeated the analysis at 155 kHz and at 97 kHz where we found that individual profiles were reliable to ± 10 s and ± 17 s, respectively. These are only estimates, of course, as we have not done an exhaustive analysis, but they are representative of the inherent uncertainty in determining the rotation period by aligning longitude profiles. Our error analysis² is sensitive to these large shifts, however, because it encompasses the entire span of data.

Second, we question the practice of combining results from several different frequencies to improve the precision of the determination. While this would be an acceptable statistical procedure in other circumstances, we have determined that the radio bursts are well correlated over a wide frequency range, and are never completely uncorrelated. Thus, receiver channels within the radio emission band contain non-independent, correlated information that cannot be averaged together, as was done by Carr et al., to reduce statistical fluctuations. We believe that conservative adherence to statistical principles would dictate that only one completely independent rotation period determination is possible using a single PRA data set.

Finally, and perhaps most importantly, the longitude profiles of Carr et al. were generated in a coordinate system locked to the Saturn-spacecraft line. However, because Voyager-1 moved 6° relative to the Saturn-Sun line during the 7-month analysis interval in question, the data should instead be organized in fixed (sub-

solar) coordinates. This angular shift will manifest itself in the longitude profiles, and hence in the rotation period, and should be taken into account. In short, given the inherent imprecision of the histogram method, the Carr et al. determination is certainly not statistically inconsistent with ours.

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CARR ET AL. REPLY—The strongest objection of Kaiser and Desch to our results is essentially that we used histograms plotted as a function of the saturnian central meridian longitude of the spacecraft instead of that of the Sun in our cross-correlations. When our oversight is corrected, we find our three rotation period measurements to be only 1 s less than they were before, and their average to be 3 s instead of 2 s less than the SLS value. This small correction does not change the situation materially.

We agree that the SLS rotation period measurement is probably more accurate than ours, but we do not believe that the difference in accuracy is large. The agreement to within 3 s increases confidence in both determinations. Our accuracy can certainly be improved by a better choice of data interval for the first histogram in each pair. We do not agree with the contention of Kaiser and Desch that little is to be gained by using data from several channels instead of just one. The redundancy resulting from the high correlation between pairs of closely spaced channels indeed tends to reduce their individual contributions to the overall precision, but is beneficial in other ways such as minimizing the effects of data gaps and of interference incorrectly identified as Saturn. The best method, in our opinion, would be to calculate the rotation period using data from each of about 10 selected channels separately by the method of Desch and Kaiser, and then to combine these values, appropriately weighted, into a single average.

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BOOK REVIEWS

Prehistoric geometers?

J.N. Graham Ritchie

STONE circles and standing stones have perhaps a greater fascination than most other classes of prehistoric monument, not because we understand more about them, but because we know less. With hill-forts, burial mounds and hut-circles we can readily appreciate their possible role within society; with stone circles and standing stones this is not so, and excavation does not add greatly to an understanding of their function. The archaeologist may discover burials at the centre of a circle or cremated bone within the packing of the socket of a standing stone, but he cannot say that burial was the only function of such sites, and the suggestion that circles may have been central places for the dissemination of legal or religious lore is a woolly alternative.

The most comprehensive interpretation of many megalithic remains has been put forward by Alexander Thom in a series of papers and books resulting from years of painstaking fieldwork and observation, particularly in Scotland, Wessex and Brittany. Thom has suggested the use of a standardized unit of length in their construction and a knowledge of complex geometry in their layout. Further, he has postulated that the erection and positioning of stones and cairns was designed to allow sophisticated observation of the major celestial bodies, including the prediction of eclipses. Thom's work, together with the influence of Gerald Hawkins and J.B. White's volume *Stonehenge Decoded* (Souvenir, 1965) in which the famous circle was described as an observatory, have created a climate of popular opinion in which the concept of prehistoric scientists and astronomers is accepted without question. How can you prove, the archaeologist is asked, that such people did not exist, or are less credible than purveyors of social or legal wisdom — classes in prehistoric society that seem to be taken for granted.

Perhaps it takes a scientist to examine a potential prehistoric colleague; in this masterly volume, Douglas Heggie, a mathematician and astronomer in the Department of Mathematics at the University of Edinburgh, has explained clearly the data on which prehistoric science rests. It is as though Heggie, the teacher, has returned to prehistoric times to test the knowledge of a student of science and astronomy. The possible range of the background information that the student might have is carefully set out in non-scientific language; whether the

Megalithic Science: Ancient Mathematics and Astronomy in North-West Europe. By Douglas C. Heggie. Pp.256. ISBN 0-500-05036-8. (Thames and Hudson: 1981.) £12, \$27.50.

student possesses this knowledge is then judiciously weighed and statistically tested, but the conclusion — to the sadness I suspect of the examiner and the reader — is that he did not have many of the skills that had been attributed to him.

Heggie has set out the evidence for the units of length which may have been used in the construction of stone circles, and has explored the ways in which the sometimes deliberately eccentric ground-plans of such sites were designed. Using a rigorously

statistical approach Heggie finds little evidence for a *highly accurate* unit of length (his italics), and while accepting that Thom's classification for the shapes of megalithic sites is helpful, he questions the need for any knowledge of Pythagorean triangles and of π in their layout. Heggie's conclusions on astronomical matters are equally tentative: that, while prehistoric man knew something of the movements of the Sun, and possibly the Moon, his role was that of an observer rather than theoretician.

Such a verdict does not lessen the achievements of early man as an architect and engineer, and perhaps increases the importance of those particular aspects of his astronomical knowledge that are found to exist. Indeed our appreciation of his strengths in empirical science are increased by such careful examination; perhaps most remarkable is the orientation of the passage grave of Newgrange, County Meath, so that the rising Sun of the winter solstice bathes the rear chamber with light, not through the entrance to the tomb, but through a specially designed opening, known as the 'roof-box'. Thus respect for the many empirical skills demonstrated by the designers and builders of stone circles and alignments is not called into question by doubts about their appreciation of theoretical science or involvement in astronomical observation to a high degree of complexity.

A subject rarely discussed in archaeological literature, and not considered in detail by Heggie, is that of the interpretation of the archaeological evidence itself. Distinct classes of stone monument can be outlined, some with a long chronological span, others covering shorter periods, and still other groups, such as simple upright stones, the dates of which remain quite unknown. Archaeologically it is important to distinguish between those sites where the upright stones delimit an open area, such as most stone circles, and those where the stones have acted as the retaining kerb of a burial cairn. The complexities of megalithic sites can be demonstrated most fully in terms of structural alteration over perhaps two millennia by the recently excavated site of Temple Wood in Argyll and our knowledge of this sequence is now much greater than that outlined in this volume. Where astronomical observations have been to the highest standard (in Thom's work this is not in question), it is unfortunate that the archaeological interpretation of archaeo-



Clouded past — part of the stone circle at Callanish, Lewis, in the Western Isles.

Scottish Development Department

astronomers has not been as scrupulous — indeed some stone alignments given astronomical interpretations may be no more than the foundation stones of turf field-walls where the ephemeral materials have weathered away. The lack of scientific knowledge for which field archaeologists are so often criticized has led to a disregard for those skills of observation and site interpretation that are their own stock in trade. A reassessment of the archaeological content of Thom's work — for in the final analysis it is on the stones themselves that the hypotheses rest — would have been a valuable adjunct to Heggie's re-examination of the data.

It is important to stress that this discussion is concerned geographically with north-west Europe and chronologically with the period between about 4000 BC and 1000 BC. Considerable momentum for the study of ancient astronomy in other parts of the world has resulted from Thom's work, though not directly stemming from it, and the levels of observational astronomy practised in other societies will clearly have a bearing on what we expect

from our present vision of prehistoric Britain. Nor will all scholars view the evidence in the same way — there is no simple "standard archaeological model", no "orthodox archaeological picture" to compare with a map of the stars; most models change with new discoveries and new approaches, either imperceptibly or radically.

Few debates can have been as measured and carefully presented. Professor Thom and his son Dr A.S. Thom have outlined in clear steps and precise documentation the basis of their findings on megalithic measurements, shape, astronomy and calendrical matters. Dr Heggie has used this material and considered it in mathematical, astronomical and statistical ways, and has presented his own findings with caution and fairness. His ability to disagree without minimizing the work of others is perhaps his greatest personal contribution. □

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Virus variety for plant pathologists

Milton Zaitlin

Handbook of Plant Virus Infections: Comparative Diagnosis. Edited by Edouard Kurstak. Pp.943. ISBN 0-444-80309-2. (Elsevier/North-Holland Biomedical: 1981.) Dfl.395, \$192.75.

A GIVEN virus often causes disease in several different plant species. Thus although well over a thousand plant virus-induced diseases are known, they are caused by only several hundred viruses (350 are considered in this handbook). This situation is further clouded by the assignment of unique names to identical diseases discovered independently.

A good conceptual framework for virus classification is therefore essential. Fortunately, ten years ago a rational approach to the problem was put forward by a small group of plant virologists in which viruses were collated into 16 "affinity" groups based on their morphology, particle composition, mode of transmission and so on. Quasi-official sanction has been given to this movement by the International Committee on the Taxonomy of Viruses, which has undertaken responsibility for the classification of viruses in general. The classification is periodically reviewed, modified and extended, and every three years, after the meeting of the ICTV, the revised classification is published as a small volume.

Edouard Kurstak's handbook follows the ICTV classification scheme, providing detailed individual chapters on virus groups, each prepared by a specialist. It is an extremely useful reference work. In

addition, there is a perceptive chapter by R.I.B. Francki dealing with plant virus taxonomy in which he outlines the basis for the classification scheme.

A true "handbook" should provide as much data as possible if it is to serve as a good reference work. Unfortunately this book suffers to some degree from unevenness in the depth of information covered for the different virus groups. As might be expected, many of the specialists have tended to emphasize their immediate interests within their assigned group, and they cover the available information beyond their specialties with differing emphasis. As one extreme example, six pages of text are devoted to a detailed listing of the hosts of the hordeiviruses, whereas little information is given for some other groups with extensive host ranges (such as the potyviruses) where such information is available. The editor is not himself a plant virologist and it is understandable that he would have to rely heavily on the judgements of the individual authors as to which topics should be emphasized.

All in all, the book is excellent and would be a welcome addition to the bookshelves of agriculturists, and to plant pathologists in particular. It is especially well-produced on quality paper and contains a great deal of tabulated information and many good photographs. Certainly, I will treasure my complimentary copy. □

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Something in the air

J.E. Lovelock

Atmospheric Sulfur Deposition: Environmental Impact and Health Effects. Edited by David S. Shriner, Chester R. Richmond and Steve E. Lindberg. Pp.568. ISBN 0-250-40380-3. (Ann Arbor Science Publishers/Butterworth: 1981.) \$29.50, £17.

AS IN the workings of a Greek tragedy, there is nothing now to indicate the discomforts that lie ahead as heedlessly we burn fossil fuel and CO₂ grows ever more abundant in the air. But this is not so for the small percentage of sulphur in these fuels; of all consequences of mankind's industrial activity on the planetary environment, none are more obvious than those caused by the emission of sulphur compounds to the atmosphere. Their presence is objectionable on scales running from the stench of reduced sulphur compounds locally released, to the planetary-scale effects on climate of the Junge stratospheric sulphate aerosol. In between these lie other consequences, such as the breath-taking lethal smogs which once beset London and the translucent sulphate aerosol which obstructs the summer sunshine over whole regions. Worst perhaps is the destruction wrought by acid deposition which diminishes life in the forests, lakes and rivers of much of the Northern Hemisphere.

Atmospheric Sulfur Deposition is an unusually tight and well-edited collection of papers from a symposium held at the Oak Ridge National Laboratory in Tennessee. It moves from the consideration of the costs and benefits of controls and their legislative implementation to the little that is known about natural sources of sulphur gases in North America, and then to the emissions from the technosphere. The atmospheric chemistry, the transfer across the interfaces with the land, and ocean, the effects good and bad on man-made and natural ecosystems, all are taken into account.

It is a book for those sizeable ranks of professionals concerned with national and regional air pollution problems. These include scientists and administrators in government and industrial service, and also legislators and regulators. For this group it will provide a very credible addition to their working knowledge of the sulphur pollution problem. The book is not primarily intended for those scientists curious about the great natural cycles of the elements and who might wish to know more about the part played by sulphur. Even so, this group also might well benefit

Missing from the review of recent textbooks of invertebrate zoology (*Nature* 295, 482; 1982) was mention of Peter Calow's *Invertebrate Biology: A Functional Approach*. Published by Croom Helm, the book costs £11.95 (hbk), £5.95 (pbk).

from reading it, for it establishes the context in which so many papers on the atmosphere and on atmospheric pollution are written, a context very different from the rarefied altitudes of academic aeronomy.

The natural cycles of the elements are closely linked and it is usually difficult to consider one of them in isolation. This restriction limits the value of the papers on the natural sources, but in any case information about natural sulphur emissions is still very meagre. The presence in the environment and the release of such compounds as COS, CS₂ and alkyl sulphides has only very recently been established. To be certain of the extent of their contribution to the natural cycle of sulphur we must wait until expeditions have measured the abundance and release rates of these transient and important compounds. It is not enough to rely on the few measurements made at sites close to the northern industrial regions. To be representative of the sulphur cycle in space and time we need measurements from all parts of the planet, made so as to include the diurnal, seasonal and secular variations. It is also possible that other sulphur compounds are yet to be discovered. Indeed, the presence of methanesulphonic acid in the sulphate aerosol has been reported. This compound, an end-product of the atmospheric oxidation of biogenic sulphur gases, may be a useful indicator of the proportion of acid deposition from these sources as compared with that coming from combustion.

There is a tendency in the book to assume that the agricultural ecosystems which cover most of the land surface are "natural" and that emissions of sulphur gases now measured from the soil are the same as those from the pre-industrial and pre-agricultural ecosystems. It now seems at least possible that the contemporary releases of sulphur gases are the product of a pathology and a consequence of a surfeit of sulphur and of other nutrients. This comment applies also to the rivers, estuaries and salt marshes which are the recipients of the run-off from agricultural and urban sewage.

Truth is said to be the first casualty of war. Looking back on the parallel environmental issue of the ozone war it seems also that scientific objectivity does not flourish in the sparring which precedes legislation and regulation. Inevitably, any collection of scientific papers from a meeting about an atmospheric topic whose objectives are national rather than global will reveal some limitation of this kind. This book commendably has overcome such a tendency to parochialism; thus the European dimensions of the acid deposition problem are well represented, and an attempt is made to link with more general scientific aspects through papers on the natural sources of sulphur gases. □

J.E. Lovelock is an independent scientist.

Antibiotics at work

H.R. Perkins

The Molecular Basis of Antibiotic Action, 2nd Edn. By E.F. Gale *et al.* Pp. 646. ISBN 0-471-27915-3. (Wiley: 1981.) £35, \$78.

SECOND editions can represent a special problem for reviewers, particularly if the movement in the subject matter (or worse, in the presentation of it) has been slight. Let me say at once that any difficulties in reviewing this particular production have arisen from precisely the opposite cause, namely that the nine years separating it from its predecessor have seen great advances in ideas about how antibiotics work. Even by the exercise of what must have been miracles of compression, the authors, all members of what one might term the Cambridge School of antibiotic investigators, have only succeeded in containing their subject within some 50 per cent more pages than before. That they have done so is a great credit to them; the book remains readable and transportable, essential characteristics of any text that has not become enshrined as an encyclopaedia.

The form of presentation remains unchanged. A general introduction to the basic concepts of antimicrobial substances is followed by logical and extensive chapters centred around the major targets of action, namely microbial wall synthesis, membrane function, nucleic acid synthesis and ribosome function. A consideration of bacterial resistance to antibiotics follows and the epilogue is entitled "Perspectives". The unifying approach is very valuable to the reader, who might be anyone from an honours microbiology student with an exceptional (or heavily directed) thirst for knowledge to a research worker seeking a detailed introduction to the field. Each chapter is well illustrated with tables and figures, and has a bibliography so generous and so recent that only an aggrieved author failing to find his own gems therein could possibly complain.

Of the three volumes covering the same general field of interest that I have reviewed recently, this is the most "up-market" in intention and there is no doubt that it achieves its object admirably. It will appeal particularly to professional chemists, biochemists and microbiologists who require a clear presentation of the latest word on the interaction of antibiotics with their targets. The coverage of information that has accrued since the first edition is impressive. When one considers that, for instance, transposons were unheard-of then and penicillin-binding proteins had not been isolated or characterized, one realizes the extent of progress that has been made in our understanding of the molecular mechanisms underlying antibiotic action and microbial resistance. Whoever purchases the present volume has an exceptional opportunity to rectify any deficiencies in his fund of information.

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Only if he wishes to know about developments in the antiviral field is he likely to go away more or less empty handed, but since the authors have largely, though not by any means entirely, confined themselves to "antibiotics" in the strict sense, this particular deficiency may be easily forgiven. □

H.R. Perkins is Professor of Microbiology at the University of Liverpool.

Truncation at length

Francisco J. Ayala

Genetic Variability. By Christopher Wills. Pp.312. ISBN 0-19-857570-X. (Clarendon/Oxford University Press: 1981.) £24, \$49.50.

THE existence of inheritable variation is, as Darwin saw it, a necessary condition for evolution. The genetic variants present in a population at a given time define the evolutionary changes that are possible in the population. It has become established during the past two decades that populations of all sorts of organisms store an enormous wealth of genetic variation. In this book, Christopher Wills, a distinguished population geneticist of the University of California at San Diego, deals only in passing with the issue of *how much* variation is there, but explores in depth the *mechanisms* that account for the variation.

This is a *roman à clef*. Wills's argument is that genetic variation is maintained largely by natural selection operating according to the truncation model. We may envisage the distribution of organisms according to their phenotypic fitness. A certain proportion of the organisms will survive and reproduce. According to the truncation model, the fitness value below which no survival occurs is not absolute but depends on the actual fitness distribution. The implications of truncation with respect to many central problems of population genetics are investigated at length, and most are published here for the first time — this is undoubtedly the most significant contribution of the book. Among the important conclusions reached, one is that the gene is the unit of selection (Chapters 8 and 9). With truncation, the strength of linkage disequilibrium in outbred populations is not a function of selection but of the population size.

According to Wills, the most compelling evidence in favour of truncation selection

is negative. If the fitness effects of each locus interact multiplicatively, then the maintenance of multiple polymorphisms would require a considerable segregational load — "highly homozygous populations should be extremely unfit" (p.36). Astonishingly, on the following page Wills states that this is "contrary to observation". This ignores a number of experiments (e.g. M.L. Tracey and F.J. Ayala, *Genetics* 77, 569–589; 1974) showing that, when fitness as a whole is measured under population conditions, the inbreeding depression is enormous — sufficiently large to account for hundreds of polymorphisms even if fitness interactions are multiplicative.

Wills asserts that whether or not most genetic variation is selectively neutral is a question "impossible to resolve, since it is impossible to prove the absence of something" (p. vii). This last phrase is obviously wrong, since I can prove to the

satisfaction of any reasonable person that there is no 747 Boeing airplane in my office. Most importantly, the statement misconstrues the aims and methods of science. The neutrality theory has been extremely fertile because it makes certain predictions about genetic variation, rates of evolution and so on. If such predictions were correct, the theory would be corroborated. Natural selection might be involved, but if it could be ignored in accounting for the relevant phenomena, there would be no reason to incorporate it in our explanations. One need not take into account the craters of Venus when explaining planetary motions. The point is a substantive one: the only hypotheses and the only parameters that are acceptable in science are those that are necessary in order to account for empirical phenomena. □

Francisco J. Ayala is Professor of Genetics at the University of California at Davis.

A time and place for fire and brimstone

Peter J. Smith

Volcanoes of the World: A Regional Directory, Gazetteer, and Chronology of Volcanism during the Last 10,000 Years. By T. Simkin *et al.* Pp.248. ISBN 0-12-787478-X. (Hutchinson Ross: 1981.) \$19.75, £13.20.

PEOPLE have been compiling lists of volcanoes since at least 1650, when Varenius tabulated 27 examples. Two centuries later, Humboldt raised the total to 407; and, when complete, the IAVCEI's *Catalog of Active Volcanoes* (begun 1951) will contain details of about 900. The magnificent Smithsonian Institution volume under review here, however, lists no fewer than 1,343 volcanoes known or likely to have been active during the Holocene and 5,564 dated eruptions from about 8300 BC onwards, all arranged regionally and chronologically, respectively, in two large tables, to which are added a gazetteer and a bibliography.

Volcanoes of the World is thus the most complete and easily accessible compilation of volcanoes and volcanic activity now available, although the authors themselves hardly dare breathe the word "complete" in this context because their listings are anything but. For a start, most volcanism takes place on the sea floor and almost all of that goes unnoticed. Even on the continents, however, there are still regions in which much volcanic activity goes unrecorded; and if that can happen in a highly populated world shrunk by modern communications, how much more extensive must have been the omissions of the past. Indeed, Simkin and his colleagues demonstrate graphically that both the total number of known volcanoes and the

number of known active volcanoes have increased roughly exponentially over the past 600 years, which suggests either that the Earth is about to be overwhelmed by volcanic activity or that the perceived volcanic state of the planet is highly dependent upon reporting efficiency.

Under such circumstances, attempts to correlate known historic volcanism with other phenomena must be undertaken warily. Even so, the compilations at the heart of this volume will be a boon to a remarkably wide range of scholars quite apart from geologists. Biologists interested in colonization will find here the 96 eruptions known to have formed islands; glaciologists can easily pick out the 15 volcanoes with known subglacial eruptions; agriculturalists may like to know of the 181 volcanoes with eruptions known to have destroyed arable land; climatologists will look for those volcanoes known to have ejected particularly large amounts of ash and dust into the atmosphere; anthropologists might examine the listed eruptions known to have severely affected communities; and so on.

In short, *Volcanoes of the World* is a splendid work of considerable interdisciplinary value and will become even more so if, as the authors hope, feedback from readers and other interested parties enables some of the many gaps to be filled. For this is not a one-off job but the first step in a continuing attempt to build as extensive a file as possible on the statistical aspects of the world's volcanism. □

Peter J. Smith is Reader in the Department of Earth Sciences at the Open University, Milton Keynes, and editor of Open Earth.

François Jacob's *The Logic of Life: A History of Heredity* (reviewed as *The Logic of Living Systems* in *Nature* 251, 81; 1974) has been reissued by Pantheon Books, New York. The book includes a new preface by the author, and costs \$7.95 in paperback.

BOOKS RECEIVED

Earth Sciences

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- GEOLOGICAL MUSEUM: INSTITUTE OF GEOLOGICAL SCIENCES. The Story of the Earth. Pp.36. Hbk ISBN 0-11-884129-7; pbk ISBN 0-11-884166-1. (HMSO: 1981.) Hbk £1.95; pbk 90p.
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Biological Sciences

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General

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